The influence of oscillating dietary crude protein concentrations on milk production and nitrogen utilization in lactating dairy cows

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ABSTRACT

There is increasing public pressure on intensive dairy operations to reduce nitrogen (N) excretion into the environment, which can be achieved by adopting on-farm feeding practices that enhance the efficiency of N utilization. One such feeding strategy that has received attention is feeding diets with oscillating crude protein (CP) concentrations, and studies with finishing beef cattle and growing sheep have reported improvements in N retention when oscillating CP diets are fed compared to static CP diets. This experiment was conducted to determine: 1) the optimum frequency of oscillating dietary CP concentration (i.e., 24, 48, or 72 h); 2) the effects of feeding oscillating CP diets on feed intake, milk production and milk composition in dairy cows; and 3) the effects of feeding oscillating CP diets on ruminal fermentation characteristics (NH_3, SCFA and pH), microbial protein synthesis, ruminal outflow of nutrients and N balance in dairy cows. Eight Holstein cows (714 ± 36 kg body weight; 114 ± 15 days-in-milk at the beginning of the experiment) were used in a replicated 4 × 4 Latin square design with 30-d periods (consisting of 18 d of dietary adaptation and 12 d of sample and data collection). Treatments were a diet containing 16.5% CP fed on a continuous basis (designated STATIC), and diets containing 13.5% and 19.5% CP that were fed on an oscillating regimen at 24 (OSC-24), 48 (OSC-48), or 72 (OSC-72) h. Diets were fed twice per day as total mixed rations with a 51:49 forage:concentrate ratio. The actual CP concentrations were 17.1% for the STATIC, and 14.3 and 20.3% for the oscillating CP diets, which deviated from the target CP concentrations due to large variations in forage CP content. Dry matter intake (mean = 26.6 kg/d) and milk production (mean = 36.4 kg/d) were not affected ($P \geq 0.19$) by dietary treatment. Milk protein yield was greater ($P = 0.03$) in cows fed the STATIC diet as compared to those fed the OSC diets (1.22 vs. 1.14 kg/d). Milk urea-nitrogen tended ($P = 0.10$) to be greater (by 8%) in cows fed the OSC-48 diet compare to those fed the OSC-24 diet. Cows fed the OSC diets tended to have greater ($P = 0.05$) ruminal NH_3-N concentrations compared to those fed the STATIC diet (12.7 vs. 11.6 mg/dL). In addition, cows fed the OSC-48 diet had a greater ruminal NH_3-N concentration compared to those fed the OSC-24 ($P = 0.05$) and the OSC-72 ($P < 0.01$) diets. Although N intakes, urinary, fecal and milk N outputs were similar across dietary treatments, apparent N balance was 22.5% greater ($P = 0.03$) in cows fed the OSC diets compared to those fed the STATIC diet. Cows fed the OSC-48 diet had a 41% greater ($P < 0.01$) apparent N balance compared to those fed the OSC-24 diet. Cows fed the STATIC diet tended to have greater

omasal DM (P = 0.10) and OM (P = 0.09) flows compared to cows fed the OSC diets. No differences in apparent total-tract nutrient digestibilities were observed due to dietary treatment ($P \geq 0.19$). Nitrogen (P = 0.06) and non-ammonia N (P = 0.07) flows at the omasal canal tended to be greater in cows fed the STATIC diet compared to those fed the OSC diets. In conclusion, these results demonstrate that feeding oscillating dietary CP diets to dairy cows on a 48-h basis improves N efficiency by enhancing N retention without compromising production when compared to feeding a static CP diet or an oscillating dietary CP on a 24-h or 72-h basis. Therefore, feeding dairy cows an oscillating dietary CP regimen could potentially decrease N excretion, thereby reducing the environmental impact that intensive dairy operations have on the environment.

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LIST OF ABBREVIATIONS

AA, amino acid

ADF, acid detergent fiber

AOAC, association of official analytical chemists

APE, atom percentage unit

BW, body weight

CM, canola meal

CP, crude protein

Cr, chromium

Cr-EDTA, chromium-ethylenediaminetetraacetic acid

DM, dry matter

DMI, dry matter intake

EE, ether extract

FAB, fluid associated bacteria

FP, fluid particulate phase

FP, fluid phase

GC, gas chromatography

GIT, gastrointestinal tract

INDF, indigestible NDF

LP, large particle phase

MUN, milk urea-N

N, nitrogen

NAN, non-NH_3-N

NANMN, non-ammonia-non-microbial nitrogen

NDF, neutral detergent fiber

NH_3-N, ammonia nitrogen

NPN, non-protein N

OM, organic matter

OSC, oscillating dietary CP

OSC-24, oscillating dietary CP on a 24-h regimen

OSC-48, oscillating dietary CP on a 48-h regimen

OSC-72, oscillating dietary CP on a 72-h regimen

OTD, omasal true digesta

PAB, particle associated bacteria

PF, particulate phase

PUN, plasma urea-N

RDP, ruminally-degradable protein

RUP, ruminally-undegradable protein

SCFA, short-chain fatty acid

SP, small particle phase

TMR, total mixed ration

UT, urea transporter(s)

WOD, whole omasal digesta

Yb, ytterbium

1. GENERAL INTRODUCTION

Concerns regarding the contribution of environmental pollutants caused by animal production, especially ruminant milk and meat production, are increasing (FAO, 2006); thus, there is a need to increase the efficiency of nutrient utilization so as to minimize nutrient excretion. In Saskatchewan, intensive livestock dairy barns commonly house animals in confinement, which causes nutrients such as nitrogen (N) to accumulate, leading to pollution of the environment. When there is excessive excretion of N into the environment, it can lead to eutrophication of surface water, contamination of ground water, smog and acidification of cropland (Galloway, 2002; Dijkstra et al., 2011).

Nitrogen in the form of crude protein (CP) is an important nutrient that is used by the ruminal microflora to produce microbial protein (MP), which is a major component of metabolizable protein that is used by the ruminant for maintenance and productive functions (e.g., milk production). Milk N efficiency is a commonly used parameter in dairy cows to evaluate how efficient the animal is in converting feed N into milk N (milk N efficiency = (milk N divided by N intake) x 100). According to Chase et al. (2009), dairy cows are not very efficient in utilizing feed N because only 25 to 35% of their dietary N intake is incorporated in milk N. From the 65 to 80% total N excretions, 60 to 80% is from urine N in which urea-N is the most common form of urine N (Van Horn et al., 1996). Urinary urea-N can be rapidly degraded to ammonia (NH_3) in the presence of urease enzymes in the environment, resulting in NH_3 volatilization (VandeHaar and St-Pierre, 2006). Ammonia release into the atmosphere contributes to acid rain (VandeHaar and St Pierre, 2006), greenhouse gas emissions (Desjardins et al., 2008) and poor air quality (Burgos et al., 2007).

There are many aspects that can contribute to how efficient ruminants are in utilizing dietary CP, such as the type of feed source and the nature of the ruminal microflora. Because ruminants have the ruminal microflora to digest high-forage feed sources we tend to supply them with high-forage diets that are lower in quality to minimize feed cost. When there is a lack of energy, ruminal microbes use amino acids (AA) as their energy source which means that ruminal NH_3-H concentrations would increase (Reynolds and Kristensen, 2008), eventually resulting in higher N excretion (Lapierre and Lobley, 2001). Fermented feed contains high levels of non-protein nitrogen (NPN) because during fermentation, feed protein is degraded to NH_3-N (Broderick, 1996). This can lead to inefficient use of N towards MP since some bacterial species

prefer to use certain N sources (peptides, AA and NH_3-N) above others (Russell et al., 1992). Due to the proteolytic nature of the ruminal microbes, feed protein is excessively degraded to NH_3-N, leading to a ruminal NH_3-N pool that exceeds the microbial NH_3-N requirements (Tamminga, 1979; Jouany, 1996; Belanche et al., 2012). When ruminal microbial NH_3-N requirements are exceeded, ruminal NH_3-N absorption leads to the wastage of N as urinary urea-N.

Several approaches can be implemented on-farm to improve the efficiency of N utilization in dairy cows. One approach is to decrease the amount of dietary N fed to dairy cows, but there are some studies that have demonstrated that this approach can negatively affect milk production. Chibisa and Mutsvangwa (2013) showed that milk yield decreased by 3 kg per cow per day and milk protein yield decreased by 140 g per cow per day when dairy cows were fed diets containing 15.2% CP compared to 17.3% CP. Also, Lee et al. (2012a) observed a reduction in milk yield in dairy cows fed a diet deficient in metabolizable protein compared to a diet adequate in metabolizable protein. The loss in milk production when dietary CP level is decreased is undesirable as it reduces profitability of dairy enterprises. An alternative approach to improving the efficiency of N utilization in dairy cows could be feeding oscillating dietary CP levels. In this approach, animals are fed high and low CP diets on an oscillating basis, with diets switched over typically at 48-h intervals. By feeding oscillating dietary CP concentrations, the efficiency of N utilization (i.e., a decrease in N excretion and an increase in N retention and growth) has been improved in steers (Cole et al., 2003; Archibeque et al., 2007b) and sheep (Cole, 1999; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011). However, to my knowledge, there is only one study (Brown, 2014) that has investigated this feeding approach in lactating dairy cows. Brown (2014) showed that feeding oscillating dietary CP concentrations had no effect on DMI, N intake, milk yield and milk composition, and excretion of urinary N and fecal N (expressed as a % of N intake) compared to feeding a static CP diet. However, that study was based on a 48-h oscillating regimen which has been used in beef cattle and sheep but might not be suitable in dairy cows which have greater levels of DM intake and, therefore, ruminal digesta outflow. Also, the study by Brown (2014) did not investigate dietary impacts on ruminal fermentation and MP synthesis. Therefore, my thesis research was conducted to determine the optimum frequency of feeding oscillating dietary CP (i.e. 24, 48 or 72 h) in dairy cows and to determine the effects of feeding oscillating CP diets on

feed intake, milk production and milk composition, ruminal fermentation characteristics (NH_3, SCFA and pH), microbial protein synthesis, ruminal outflow of nutrients, and N balance in dairy cows.

2. LITERATURE REVIEW

2.1 Environmental Impacts of Excess N

Agricultural producers are currently facing challenges to feed the growing population predicted to peak at 9.2 billion people by 2050 while meeting social and environmental responsibilities to decrease N emissions (FAO, 2006). This growing world population has led to the increase in the demand for dairy products. The rise of larger farming systems as well as moving more towards intensive farming systems have led to concerns about nutrient accumulation in the environment (Van Amburgh et al., 2008). One of the nutrients of major concern is nitrogen (N). Nitrogen is excreted in ruminant urine and fecal matter.

Ruminants are not very efficient in utilizing N, as up to 80% of feed N can be lost in urine (Van Horn et al., 1996). The major form of N excretion is urinary urea-N (Van Horn et al., 1996). This poses a problem as urinary urea-N can be rapidly degraded to ammonia (NH_3) due to the presence of urease enzymes that are ubiquitous in the environment. Ammonia is rapidly volatilized into the environment contributing to acid rain (VandeHaar and St Pierre, 2006). When ammonium reacts with other atmospheric chemicals it forms particulates which cause poor air quality that leads to human health concerns (Burgos et al., 2007). Ammonia can be converted into nitrous oxide (N_2O), which is a greenhouse gas (Desjardins et al., 2008). Therefore, the excretion of N in the form of urinary urea-N is undesirable for a variety of reasons.

According to Klausner (1993), from the amount of N that is brought onto the farm (feed and fertilizer) only 21 to 38% leaves the farm as usable product (meat and milk); thus, the residual N is eventually lost into the environment (Kohn et al., 1997). Manure is a valuable product that can be spread onto cropland to increase soil organic matter, microbial biomass, and mineralization rate (Spiehs et al., 2010; Langmeier et al., 2002), thereby increasing crop yields (Khaleel et al., 1981; Araji et al., 2001). When manure is applied to cropland, N_2O may be released during nitrification and denitrification. Nitrification occurs when ammonium (NH_4^+) in the soil is converted to nitrite (NO_2^-) which is then oxidized to nitrate (NO_3^-), a precursor for nitrogen gases (such as nitrous oxide). Denitrification is a process that occurs when

microorganisms reduce nitrate to nitrogen gases (NO, N_2O, N_2) under anaerobic conditions (Banham et al 2003). Nitrogen oxide (N_2O) is a stronger greenhouse gas than CO_2 because it has a 296 times higher global warming effect; thus, 1 metric tonne of N_2O is equivalent to 296 metric tonnes of CO_2 (FAO, 2006). It is estimated that the agricultural sector contributes 20% of global greenhouse gas production; however, in Canada the agricultural sector is estimated to contribute 10% of Canadian greenhouse gas emissions which is comprised of 47% from animal production, 32% from crop production and 21% from on-farm fuel use (Environment Canada, 2013). In 2001, N_2O contributed 36% of the greenhouse gas emissions from the Canadian beef industry (Desjardins et al., 2008).

Because of the impacts of intensive ruminant production systems like dairy farms on the environment, there is increasing pressure from the public and governments to reduce nutrient excretion into the environment. Thus, there is a need to develop feeding strategies that can increase the efficiency of nutrient (N) utilization in ruminants, which would minimize their environmental footprint.

2.2 Rumen nitrogen metabolism

2.2.1 Protein Requirement

Ruminants have the ability to utilize N feed sources for growth, maintenance, and productive functions, unlike non-ruminant animals that require feed sources containing amino acids (AA) for these functions. The microbial population within the reticulo-rumen is responsible for the degradation of feed CP, yielding N compounds (peptides, AA and NH_3-N) that are available for the synthesis of MP (Bach et al., 2005). Eventually, the microbes are washed out of the rumen and end up in the small intestine where they are degraded into AA. These AA are absorbed and ultimately utilized by the ruminant for growth, maintenance and production functions. In addition, some of the AA absorbed in the small intestine are derived from ruminally-undegradable protein (RUP) that escapes ruminal degradation as well as endogenous protein that is composed mainly of sloughed-off epithelial cells, enzymes and glycoproteins of mucus (Tamminga, 1992). Collectively, MP, RUP and, to a lesser degree, endogenous proteins that are digested at the small intestine with the resulting AA being available for absorption are referred to as metabolizable protein (NRC, 2001). Microbial protein produced in the rumen is a major component of metabolizable protein, providing 50-80% of the metabolizable protein

requirements of high-producing dairy cows (NRC, 2001). The AA profile of MP is very similar to the AA profile of meat and milk (NRC 2001), thus MP is a high quality protein that provides the ideal profile of essential amino acids for meat and milk protein synthesis. Not all of the N in the feed is used towards MP synthesis as some N is absorbed as NH_3-N into the blood, detoxified in the liver through ureagenesis, with the resulting urea being excreted in the urine. Due to a better understanding of how feeds are degraded in ruminants, ruminant diets nowadays are formulated to meet the metabolizable protein requirements of productive ruminants, rather than being formulated on dietary CP content alone.

Ruminants have the ability to utilize ruminally-degradable protein (RDP) from feed sources by having ruminal microflora that synthesize AA from the degradation of RDP. Due to the presence of ruminal microflora, ruminants have the ability to consume poor quality feed and convert it into high quality food products for human consumption. There are three sources of N in the rumen for microbial use. The first N source is dietary CP that includes non-protein N (NPN) and true protein, with the true protein being comprised of RDP and ruminally-undegradable protein (RUP; Bach et al., 2005). Degradation of RDP yields peptides, AA and NH_3-N (Bach et al. 2005). Ruminally-undegradable protein flows out of the reticulo-rumen directly to the small intestine where it can undergo digestion, with the resulting AA being available for absorption. Ruminally-undegradable protein supply can be beneficial for high-producing animals as MP alone cannot meet their metabolizable protein requirements. The AA from RUP can complement the AA from MP, which contributes to a more efficient supply of AA to the animal (Owens and Zinn, 1988). Nitrogen in nucleic acids, NH_3-N, AA, small peptides, amides and amines are classified as NPN. Non-protein N is extensively broken down to NH_3-N (Smith, 1989), which is available for utilization by most ruminal bacteria for bacterial protein synthesis (Bach et al. 2005). The second source of N is recycled urea-N that can enter the rumen through salivary secretions or directly from the bloodstream across the ruminal epithelium (Stewart et al., 2005). The third source of N is endogenous N, which comprises of sloughed-off epithelial cells, enzymes, and glycoproteins of mucus (Bach et al., 2005).

2.2.2 Ruminal Protein Degradation and Microbial Protein Synthesis

The majority of ruminal microbes have proteolytic activity (Prins et al., 1983), where the majority of it occurs in the particulate phase of the ruminal digesta (Brock et al., 1982).

Prevotella spp., Butyrivibrio sp., Ruminobacter sp., and *Selenomomas sp.* are the main proteolytic bacteria species within the rumen (Prins et al., 1983). Ingested feed moves to the rumen where microbes adhere to the feed particles (Brock et al., 1982). Ruminal bacteria then release different cell-associated and extra-cellular proteases that includes dipeptyl aminopeptidases and aminopeptidases which, in combination, are necessary to degrade a large number of various types of structures of the dietary protein to peptides, AA and NH_3-N (Wallace, 1996). Dietary protein structure and chemistry can affect protein degradation as well as ruminal pH and the predominant microbial species present within the rumen (Huntington and Archibeque, 2000). There are some AA and peptides that get washed out of the rumen (Chen et al., 1987), but only contribute a diminutive amount of metabolizable protein (Leibholz, 1971; Martens et al., 2001).

After degradation, the AA and peptides are then moved into the bacterial cell (Tamminga, 1979). Oligopeptides are released from the hydrolysis of protein by bacteria within the rumen; *Prevotella ruminicola* is the main species that is responsible for cleaving off oligopeptides from protein (Wallace and McKain, 1991). Dipeptides are cleaved off from the N-end of the oligopeptide, by using intracellular dipeptidyl peptidases. This process continues until only dipeptides and AA are leftover. Single AA is also cleaved off from the N-end of the oligopeptide, by using aminopeptidases, *S. bovis* and *R. amylophilus* are the main species responsible for this process (Wallace and McKain, 1991). Dipeptidases are used by *P. ruminicola*, *Meldenii*, *F. succinogenes*, and *L. multiparas* to cleave off AA from dipeptides and tripeptides (Wallace et al., 1996). Bacteria incorporate AA into MP but can also deaminate AA; *P. ruminicola* plays a role in AA deamination. It has been shown that some types of microbes have preferation to which AA they degrade whereas others have been shown to degrade all AA (Scheifinger et al., 1976). Peptide contribution to bacterial N requirement is only 11% to 35%, whereas AA contribution is 6% to 68% (Armstead and Ling, 1993). Ammonia-hyperproducing gram-positive bacteria (e.g., P *anaerobius*, *C. sticklandii*, and *C. aminophilum*) is a small consortium of bacteria in the rumen which contains deaminase activity that contributes significantly to ammonia production (Attwood et al., 1998). Epimural bacteria have high proteolytic activity and are responsible for recycling protein from sloughed epithelial cells (Hobson and Stewart, 1997) as well as hydrolysing the urea-N entering through the rumen epithelium (Krause et al., 2003). In the presence of energy, NH_3-N, peptides and AA are utilized

for the synthesis of MP. Some bacterial species have a preference for which N source they utilize for protein synthesis. For example, *Streptococcus bovis* can utilize peptides, AA and NH_3-N, whereas *Fibrobacter succinogenes* prefer NH_3-N (Russell et al., 1992). Amino acid deamination occurs when energy is restricted, resulting in the production of SCFA, CO_2, CH_4 and NH_3-N (Tamminga, 1979).

Ruminal bacteria are not the only microorganisms responsible for protein degradation. Protozoa (e.g. *Entodinium spp*. and *Holotrichs*) enzymatically degrade engulfed dietary insoluble protein molecules, as well as bacteria, to peptides, AA and NH_3-N (Tamminga, 1979; Jouany, 1996; Belanche et al., 2012). Proportions of the AA are used to synthesize protozoal protein because protozoa cannot synthesize AA from simple N compounds (e.g. NH_3-N) like bacteria (Jouany 1996). The leftover AA and NH_3-N are excreted out of the protozoal cell where they become available for bacteria to use towards the synthesis of bacterial protein (Belanche et al., 2012). Protozoa survive in the rumen by associating with the particle phase of ruminal digesta (Williams and Coleman, 1997). Without this survival mechanism, protozoa would be washed out of the rumen due to having longer generation time (6 to 60 h) and slower turn-over rate compared to bacteria (Jouany, 1996). Due to their slower passage rate, protozoa contribute little to available metabolizable protein to the host, although protozoa contribute 20 to 70% of the total ruminal microbial biomass (Jouany, 1996). Protozoa contribute to N recycling in the rumen by extensive degradation of bacterial, dietary and endogenous proteins, therefore increasing ruminal NH_3-N levels. Elevated ruminal NH_3-N levels lead to increased absorption of NH_3-N into portal blood, thereby resulting in an energetically-costly detoxification process where urea-N is produced from NH_3-N in the ornithine (urea) cycle. Ruminal anaerobic fungi (e.g., *Neocallimastix frontalis*) have limited proteolytic activity (Wallace, 1996).

2.2.3 Ammonia Absorption, Ureagenesis and Urea-Recycling

The degradation of dietary true protein, NPN, endogenous N sources and protozoal metabolism all contribute towards the NH_3-N pool within the rumen (Bach et al., 2005). When urea-N enters the rumen, it is rapidly degraded to NH_3-N and CO_2 by bacterial urease (Stewart et al., 2005). There are three major pathways for NH_3-N to leave the rumen: the first one is for NH_3-N to be incorporated into MP; the second is outflow from the rumen, which is dependent on the outflow rate of ruminal fluid; and the third is absorption of NH_3-N through the ruminal

epithelium (Siddons et al., 1985). There are two ways of NH_3-N absorption across the ruminal epithelium, which are dependent on the form of NH_3-N. When NH_3-N is in the unionized form (i.e., as NH_3, which is more lipid soluble), it can freely diffuse down a concentration gradient into the bloodstream (Hogan, 1961). On the other hand, when it is in the ionized form (i.e., as ammonium [NH_4^+]) which is less lipid soluble, it is transported across the epithelium with the help of potassium channels in the ruminal epithelium (Bödeker and Kemkowski, 1996, Rémond et al, 1996). Ammonium is the predominant form when the ruminal pH is between 6 and 7. Ammonium dissociates into NH_3 and H^+ after absorption. It has been suggested that some transport proteins might be involved with NH_4^+ absorption and that chemical and electrical gradient might be regulators for NH_4^+ movement across the ruminal epithelium (Abdoun et al., 2007).

Once NH_3 (toxic) is in the blood it is transported to the liver, detoxified in the periportal liver cells by converting it into urea-N (Haussinger, 1983; Haussinger et al., 1992). There is evidence that NH_3-H can be converted into urea-N in the rumen epithelial cells and duodenal mucosal cells (Oba et al., 2004). In order to detoxify NH_3-N, ATP and HCO_3^- is required, forming carbamoyl-phosphate which then enters the ornithine-urea cycle (Meijer et al., 1990). Once urea-N is formed it can be recycled back to the gastrointestinal tract (GIT) or excreted in the urine.

In unfavorable nutritional conditions, ruminant animals have a survival mechanism referred to as urea-N recycling that allows them to recapture N that would otherwise be irreversibly lost as urinary urea-N. With this mechanism, urea-N is recycled back to the GIT either through saliva or direct transfer from the blood through the GIT epithelium (Reynolds, 1992; Stewart et al., 2005; Reynolds and Kristensen, 2008). This mechanism provides the GIT microbes with NH_3-H which they can use for MP synthesis that provides the ruminant with a high quality protein source (Lapierre and Lobley, 2001). The one organ that plays a big part in urea-N recycling is the kidney. The kidney has specific mechanisms that control the excretion or retention of urea, which depends on the ruminant's N requirements (Harmeyer and Martens, 1980). To recycle urea-N back to the GIT when ruminal NH_3-N concentrations are low due to low feed N intake, renal plasma flow and glomerular filtration rates would decrease, inner medullary collecting duct urea-N re-absorption would increase and a higher BUN concentration would be maintained (Tebot et al., 2002).

Urea transporters in the cells of the kidney, liver, reticulum, parotid gland, small intestine, colon and rumen epithelium are responsible to mediate urea-N flux across cells (Bagnasco, 2005; Ludden et al., 2009; Smith, 2009; Stewart, 2011, Klein et al., 2012). There are two main urea-N transporters namely UT-A and UT-B that can be further categorized into more specific transporters namely UT-A1, UT-A2, UT-A3, UT-A4, UT-A5, UT-A6, UT-B1 and UT-B2 (Smith et al., 1995; Couriaud et al., 1996; Shayakul et al., 1996; Tsukaguchi et al., 1997; Karakashian et al., 1999; Fenton et al., 2000; Smith et al., 2004). UT-B2 is the major transporter found in ruminal epithelia which allows cell to cell urea-N transport (Stewart et al., 2005). Hence, trans-epithelial urea-N transport was suggested to be mediated by UT-B. Since the discovery of the urea transporters many studies have investigated UT-B expression in ruminal epithelium of cattle, sheep and goats and how dietary CP content and fermentable carbohydrates affect UT-B expression (Marini and Van Amburgh, 2003; Marini et al., 2004; Ludden et al., 2009; Simmons et al., 2009; Muscher et al., 2010; Røjen et al., 2011). Discrepancies about how dietary N content affect ruminal UT-B expression have been reported by various groups. Marini and Van Amburgh, (2003), Ludden et al. (2009) and Simmons et al. (2009) found that ruminal UT-B expression was up-regulated when dietary N content increases. In contrast, Marini et al. (2004), Muscher et al. (2010) and Røjen et al. (2011) found no effect on UT-B expression when dietary N content increased. It has been found, in vitro, that mRNA and protein abundance for UT-B in ruminal epithelium were stimulated by low pH and the presence of SCFA (Lu et al., 2015). Ammonia has the opposite effect of SCFA and low pH. It is clear that there is more than one variable that can affect urea-N recycling and, therefore, more research needs to be done looking at how multiple variables together affect urea-N recycling.

2.3 Factors That Regulate Urea Recycling

2.3.1. Dietary N Supply

The amount of N recycled back to the rumen is dependent on a variety of factors (e.g. dietary N intake, ruminal pH). When low dietary CP diets are fed to ruminants more urea-N is delivered to the GIT (Ford and Milligan, 1970; Owens and Bergen, 1983; Leng et al., 1984; Isozaki et al., 1994; VanSoest, 1994). It has been speculated that the increase in urea-N recycling to the GIT might be due to increased activity of the urea transporters in the GIT epithelia (Ritzhaupt et al., 1997). High ruminal NH_3-N concentrations from high dietary CP content

causes the use of recycled N for MP production to be less efficient (Castillo et al., 2001). Approximately 43% of the N recycled back to the rumen was used for MP production when heifers were fed a low dietary CP diet compared to 6% when a high dietary CP diet was fed (Marini and Van Amburgh, 2003). Total endogenous urea-N production as a percentage of N intake can range from 77 to 95% (Lobley et al., 2000). When the effect of N intake on N recycled to the GIT in lambs was investigated by Marini et al. (2004), it was found that the lambs fed the low N diet had greater urea-N recycled to the GIT as a percentage of N intake than the lambs fed the high N diet; however, as dietary N intake decreased, urea-N recycled to the GIT decreased as well. It would be unproductive if urea-N is recycled back to the rumen when ruminal NH_3-N concentrations are too high because the recycled urea-N would be hydrolyzed to NH_3-N. This NH_3-N would just be reabsorbed and detoxified in the liver again because the NH_3-N from the diet would be sufficient for MP synthesis. This leads to extra energy waste by the animal. This led to the belief that ruminal NH_3-N concentrations regulate urea-N recycling back to the rumen. When NH_4Cl was infused into the rumen of sheep, it caused urea-N recycling to the rumen to decrease (Norton et al., 1982). Many studies have confirmed this (Ford and Milligan, 1970; Kennedy, 1980; Leng et al., 1984; Isozaki et al., 1994). There is a negative correlation between ruminal NH_3-N concentration and urea-N recycled back to the rumen (Kennedy and Milligan, 1980). Urease activity is also negatively correlated to ruminal NH_3-N concentration (Cheng and Wallace, 1979).

Another factor that affects the extent of urea-N transfer to the GIT is plasma urea-N (PUN) concentration. There is a positive correlation between PUN concentration and urea-N transfer (Kennedy and Milligan, 1980). Plasma urea-N is highly correlated with ruminal NH_3-N concentration (Thornton, 1970; Hammond, 1983a; Hennessy and Nolan, 1988). When urea was infused into the blood of sheep fed a low N diet, PUN increased leading to an increase in urea-N transfer to the GIT (Sunny et al., 2007). When dietary CP content fed to goats was decreased, an increase in urea transport was observed when ruminal epithelium was mounted in Ussing chambers (Muscher et al., 2010). They also observed that when PUN concentrations were below 1.75 mmol of urea/L, serosal-to-mucosal urea flux rates increased but when PUN concentrations were higher than 1.75 mmol of urea/L of plasma it did not affect urea flux. This clearly indicates that low PUN do stimulate urea-N transfer to the rumen; however, the mechanisms are still unclear.

The type of protein fed to the animal can also play a role in urea-N recycling. It is observed that ruminal NH_3-N concentration was greater when cows were fed high RDP diets compared with those fed low RDP (Cunningham et al. 1996; Reynal and Broderick 2005). This means that type of protein source does not directly influence urea-N recycling, but it does so by influencing ruminal NH_3-N concentration which is negatively correlated with urea-N recycling.

2.3.2. Supply of Ruminally Carbohydrate Fermentation

Ruminal microbes use carbohydrates as an energy source. When dietary carbohydrate (energy) content increases, more ruminal energy is available for the microbes to use towards MP synthesis which leads to decreased ruminal NH_3-N concentration. As discussed previously, decreased ruminal NH_3-N concentrations stimulate urea-N recycling (Kennedy, 1980; Kennedy and Milligan, 1980; Huntington, 1989; Huntington et al., 2009). In the presence of energy, ruminal microbe's capture peptides, AA and NH_3-N for the synthesis of MP (Nocek and Russell, 1988). Lapierre and Lobley (2001) observed that when a diet is supplemented with a readily fermentable carbohydrate source, dietary N and endogenous N captured by ruminal microbes increase which led to an increase in MP reaching the small intestine compared to a diet that was not supplemented. Similarly, when sheep (Henning et al., 1993) and non-lactating cows (Kim et al., 1999) were infused with an energy source microbial N output were higher than when no energy were infused. When Sairanen et al. (2005) supplemented lactating cows with a grain-based concentrate, ruminal NH_3-N concentration, MUN and urinary N excretion decreased linearly, MP, milk protein content and yield increased linearly suggesting that N utilization increased. Supplementing starch to steers fed gamagrass- or orchardgrass-based diets resulted in a decrease in urea-N synthesis, as well as a decrease in urinary urea-N excretion (Huntington et al., 2009). Thus, the urea-N recycled to the GIT was incorporated into MP and led to increased N retention (Huntington et al., 2009).

When Marini and Van Amburgh (2003) fed heifers, isocaloric diets with different N contents (1.89, 2.50, and 2.97% N) they observed a decrease in renal urea-N clearance and an increase in GIT urea-N clearance and MP from recycled urea-N. However, they did not observe an effect on the amount of urea-N recycled back to the GIT or total MP yield. In contrast, when Sarraseca et al. (1998) looked at the effect of different levels of N intake in sheep they found that when N intake decrease, total and % of hepatic production urea-N recycled back to the GIT

decrease. The amount of urea-N returned to the ornithine cycle was constant. In this study the different N intakes were accomplished by having 3 feed restricted levels (0.6 1.2 and 1.8 times estimated maintenance energy intake). Thus, DMI was not the same which means that the energy to protein ratio were constant for the 3 intake levels. These results suggest that energy can regulate urea-N recycling. Other authors (Kennedy and Milligan, 1980; Huntington, 1989; Theurer et al., 2002) also found that when dietary energy increase, urea-N recycled back to the GIT increase. It has been reported that concentrate diets fed to ruminants stimulate mRNA and protein expression of UT-B in ruminal epithelium (Simmons et al., 2009; Lu et al., 2015). This might be the reason for the increase in urea-N recycled back to the GIT that have been reported (Kennedy and Milligan, 1980; Huntington, 1989; Theurer et al., 2002)

Changing ruminal fermentability of feed sources through processing can also impact urea-N recycling (Kennedy and Milligan, 1980; Huntington, 1989; Alio et al., 2000; Delgado-Elorduy et al., 2002; Gozho et al., 2008). When dairy cows were fed barley grain that was rolled (high fermentable), urea-N recycled back to the GIT tended to be higher than when the cows were fed barley grain that was pelleted (low fermentable; Gozho et al., 2008). When growing beef steers were fed sorghum grain that was dry-rolled (lower fermentable), increased splanchnic output of urea-N were observed than compared with steers that were fed sorghum grain that was steam-flaked (higher fermentable; Alio et al., 2000). This means that absorbed N could have been potentially conserved. Similarly, Delgado-Elorduy et al. (2002) reported that urea-N recycled back to the GIT doubled when steam-flaked corn (higher fermentable) compared with steam-rolled corn (lower fermentable) were fed to dairy cows. The authors reported that despite the increase in urea-N recycling to the GIT there was no increase in MP flow to the small intestine, AA uptake by the mammary tissues increase by 56% when cows were fed steam-flaked corn compared to steam-rolled corn.

The effect of the change in SCFA profiles in the rumen on urea-N recycling has led to mixed results. When wethers fed orchardgrass hay were ruminally infused with butyrate, urea-N transfer into the rumen decreased 29% (Remond et al., 1993). In contrast, Norton et al. (1982) observed an increase in urea-N transfer when butyrate was infused in the rumen of sheep. A four-fold increase in urea flux into the rumen was observed when ruminal epithelium that was mounted in Ussing chambers in the presence of SCFA (Abdoun et al., 2009). When steers were fed a concentrate diet, the expression of UT-B in ruminal tissue was increased compared to those

fed a silage diet (Simmons et al., 2009). Lu et al. (2015) observed that when SCFA and low pH are combined it stimulates mRNA and protein expression of UT-B in ruminal epithelium in vitro. When the pH was 6.8 and NH_4Cl was present mRNA and protein levels of UT-B decreased.

2.3.3. Efficiency of Nitrogen Utilization in Dairy Cows

Ruminant animals are not very efficient in utilizing feed N towards growth, maintenance and productive functions. There are some parameters that can be used to evaluate the protein status of the animal as well as N utilization efficiency. Plasma urea-N is one of the parameters that can be used since it is highly correlated with ruminal NH_3-N concentration (Thornton, 1970; Hammond, 1983a; Hennessy and Nolan, 1988). In dairy cows, MUN can be used to monitor N utilization efficiency because MUN is highly correlated with PUN as PUN freely diffuses from the blood into the milk (Roseler et al., 1993; Baker et al., 1995; Butler et al., 1996). Milk N efficiency, which is calculated as milk N divided by N intake x 100, can also be used to evaluate N utilization efficiency. It has been observed that milk N efficiency can range from 20 to 35% in dairy cows (Chase et al., 2009); consequently, this means that 65 to 80% of feed N is lost in urine and feces. Urine N excretion can account for up to 60-80% of total N excreted by the animal (Van Horn et al., 1996). It has been observed that beef cattle only retain 9-33% of feed N; consequently, 21-55% of feed N is lost in urine and 26-48% of feed N is lost in feces (Bierman, 1999). Urine urea-N can range from 50 to 90 % of urinary N (Van Horn et al., 1996; Reynal and Broderick 2005). Overall, dairy cows are usually more efficient in utilizing feed N than feedlot cattle because dairy cows use feed N towards milk and N retention, whereas feedlot animals only use feed N towards N retention (Bierman, 1999).

When low dietary CP diets are fed to ruminants, more urea is delivered to the GIT through recycling (Ford and Milligan, 1970; Owens and Bergen, 1983; Leng et al., 1984; Isozaki et al., 1994; VanSoest, 1994) and, thereby, N can be more efficiently used. When a lower dietary CP content (15.2% CP) was fed to dairy cows compared to a higher dietary CP content (17.3% CP), a 3 kg/d drop in milk yield was observed as well as a 140 g/d drop in milk protein yield (Chibisa and Mutsvangwa, 2013). This negative effect on milk production is undesirable. To compensate for the loss of milk, more cows would be needed resulting in more N excreted. Raising dairy cow herd productivity can decrease environmental N excretion. St-Pierre and

Thraen, (1999) observed that lower producing cows excrete less N overall but a higher amount of manure N per kg of milk was excreted.

Increasing carbohydrates in the diet lead to an increase in urea-N recycling to the rumen (Huntington, 1989; Rémond et al., 1996) which means that more NH_3-N is available for the synthesis of MP. Shifting fermentation site (rumen vs hindgut) can result in higher fecal N, resulting in less N volatilization. Theurer et al. (2002) showed that site of carbohydrate digestion can be shifted by using different grain processing methods. Steam-flaked sorghum grain would be digested in the rumen whereas dry-rolled sorghum grain would be digested in the small intestine. When dry-rolled sorghum grain is fed more N would be recycled back to the hindgut where it would become available for MP synthesis. These microbes would be excreted in the manure, decreasing N in the rumen and urine, but not total N excreted. This method is not desirable because the N is not utilized by the animal. There are many factors that can influence how efficient ruminants are in utilizing feed N.

2.4 Strategies to Increase the Efficiency of N Utilization in Ruminants

2.4.1. Dietary N Supply

Overfeeding dietary CP can be expensive because the increased CP content in the feed does not always yield an increase in milk production. Besides, when dietary CP increases it usually increases urinary N excretion (Broderick, 2003), which leads to increases in environmental pollution. On the other hand, underfeeding dietary CP can also be expensive due to a decrease in milk yield as well as milk protein yield, which leads to decreased revenue (VandeHaar and St-Pierre, 2006). High dietary CP diets increase ruminal dietary CP degradation which leads to elevated ruminal NH_3-N concentrations and, ultimately, result in N wastage due to NH_3-N absorption from the rumen (Hristov et al. 2004; Olmos Colmonero and Broderick, 2006b; Chibisa and Mutsvangwa, 2013). This has led to studies that have been conducted to investigate the effects of decreasing dietary CP on N efficiency and milk production

Broderick (2003) investigated the effect that three dietary CP levels (15.1, 16.7 or 18.4%) with three different dietary energy levels would have on milk yield in dairy cows. Milk yield and milk protein yield increased when the dietary CP content was increased from 15.1 to 16.7% when the low or medium energy levels were fed but not when the high energy level was fed. Increasing the dietary CP content above 16.7% resulted in no effect on milk yield or milk protein

yield. When dietary energy levels increased, milk yield and milk protein yield increased. Decreasing the dietary CP content increased the milk N efficiency. In that study, when dietary CP decreased from 18.4 to15.1% total urinary N and urinary urea-N excretion decreased by 69 and 82%, respectively. Similarly, Olmos Colmonero and Broderick (2006b) investigated the effect that five dietary CP levels (13.5, 15.0, 16.5, 17.9 or 19.4%) would have on milk production as well as N excretion in dairy cows. Dry matter intake, milk yield and milk protein content were not affected by diet, but there was a tendency for a quadratic response in milk yield. Milk fat content increased when dietary CP content increased. The increase in dietary CP content caused ruminal NH_3-N concentrations to increase which, ultimately, led to an increase in urinary N as well as urinary urea-N excretion. The authors also observed that milk N efficiency decreased from 36.5 to 25.4% as dietary CP content increased from 13.5 to 19.4%. Other studies also reported that feeding low to medium CP diets decreased ruminal NH_3-N concentrations, PUN and N excretion compared to feeding high CP diets (Hristov et al. 2004; Lee et al., 2011b; Chibisa and Mutsvangwa, 2013; Hristov and Giallongo, 2014). Many studies indicated that by decreasing dietary CP content in the last phase of feedlot cattle before slaughter had no impact on animal performance (Putnam et al., 1969; Dartt et al., 1978; Cooper et al., 2000; Klopfenstein and Erickson, 2002; Cole et al., 2006; Cole and Todd, 2008).

When dietary CP content was decreased from 17.3 to 15.2% CP, milk yield decreased by 3 kg per cow per day and milk yield protein decreased by 140 g per cow per day (Chibisa and Mutsvangwa, 2013). Additionally, in that study total N excretion also decreased when dietary content was decreased from 17.3 to 15.2% CP. However, the loss in production is undesirable when low CP diets are fed and a better solution for decreasing N excretion while maintaining production is key. Achieving high milk yield and milk protein yield in dairy cows can be accomplished by supplementing lower CP diets with ruminal protected AA. Most feed sources are deficient in lysine and methionine (NRC 2001). Thus, supplementing ruminally-protected lysine and methionine can increase or maintain milk yield when dietary CP content are lower (Armentano et al., 1997; Dinn et al., 1998). Lee et al. (2012a) investigated the effect of supplementing metabolizable protein-deficient diets with ruminally-protected AA (lysine, methionine and histidine) on milk production and N excretions. In that study, there were four diets that were tested as follows: a metabolizable protein-adequate diet (MPA; 15.7% CP); a metabolizable protein-deficient diet (MPD; 13.5% CP); a metabolizable protein deficient-diet

supplemented with lysine and methionine (MPDLM); and a metabolizable protein-deficient diet supplemented with lysine, methionine and histidine (MPDLMH). The authors reported that DMI and milk yield were lower (by 1.5 kg/d and 3.6 kg/d, respectively) when the MPD diet were fed to cows compared with the MPA. Clearly the reduction in DMI resulted in a decrease in milk yield. By feeding the MPD to cows the urinary urea-N were decreased compared to when MPA were fed (Lee et al., 2012a). Compared with MPA, DMI and milk yield was similar when the MPDLM and MPDLMH diets were fed to cows. In that study, urinary N as well as urinary urea-N excretion decreased when the MPDLM and MPDLMH diets were fed to cows compared with the MPA diet. From this study it is clear that by supplementing metabolizable protein-deficient diets with ruminally-protected lysine, methionine and histidine can maintain milk production while improving environmental stewardship. Similarly, Giallongo et al. (2015) reported that milk true protein content and yield increased when cows were supplemented with methionine and histidine in metabolizable protein-deficient diets compared with supplementation with methionine alone. Thus, diets that are low in CP can be supplemented with ruminally-protected AA to decrease N excretions while maintaining milk production. The on-farm adoption of this approach, however, is limited because the high cost of ruminally-protected AA would increase feeding costs.

2.4.2. Nitrogen Source

It is important to balance the dietary supply of RUP and RDP to meet the metabolizable protein requirements of the ruminant. If this is achieved without overfeeding dietary CP it can lead to not only positive effects on feed cost but also on the environment (Kalscheur et al., 1999). It is clear that milk yield increases when dietary CP concentrations are increased in the diet of high producing cows (>30 kg milk/cow/day; Grings et al., 1991; Powers et al., 1995; Komaragiri and Erdman, 1997; Kalscheur et al., 1999; Broderick, 2003); however, there is a point where each extra unit of dietary CP would not yield an increase in milk yield (Metcalf et al., 2008). As already discussed above (Section 2.4.1), dietary supplementation with ruminally-protected AA when low dietary CP diets are fed can increase milk yield while reducing N excretion which, consequently, would improve environmental stewardship.

The type of protein fed to the animal can also play a role in altering ruminal NH_3-N concentrations and, as a result, N excretion. When cows were fed high RDP diets they had

elevated ruminal NH_3-N concentration compared with those fed the low RDP diets (Cunningham et al. 1996; Reynal and Broderick 2005). Belanche et al. (2012) observed greater ruminal NH_3-N concentrations when cows were fed a diet that met 110% of the RDP requirement compared with a diet that met 80% of the RDP requirement. Similarly, Hristov et al. (2004) reported that ruminal NH_3-N concentrations and urinary N excretion increased and milk N efficiency decreased when a high RDP diet was fed to dairy cows compared with a low RDP diet. Protein sources can be processed differently to change the degradability of the protein. When the oil is processed out of SBM by a heat-generating expeller processor (Borucki Castro et al., 2007) or treated with lignosulfate, (Can and Yilmaz, 2002), the RUP fraction can increase. Increasing the RUP fraction can result in higher protein supply to the small intestine which will result in decreased N excretion. When a high RDP (raw pea) diet was fed to sheep, ruminal NH_3-N losses were higher than when a lower RDP (extruded pea) diet was fed (Rémond et al. 2009).

The degradation rate of RDP can also play a role in N utilization. Galo et al. (2003) reported that by feeding a diet to dairy cows that contains a slow degradable RDP have less fluctuations in ruminal NH_3-N concentrations compared with a rapidly degradable RDP diet which ultimately decrease urinary urea-N excretions. This means that the protein degradation rate can directly influence ruminal NH_3-N concentration that is negatively correlated with urea-N recycling and thereby effects N excretions. Soybean meal (SBM) and canola meal (CM) are both regularly used in dairy diets, with SBM having a greater CP concentration than CM (49.9 vs. 37.8% CP, respectively; NRC, 2001). Huhtanen et al. (2011) reported that SBM supplies a higher metabolizable protein compared with CM on a similar CP basis, which means that in order to supply the same amount of dietary metabolizable protein a higher dietary CP content should be fed when using CM as protein source.

Ruminal NH_3-N concentrations, PUN and MUN were lower when cows were fed an alfalfa silage diet supplemented with an AA N source compared with those supplemented with a urea-N source (Broderick et al., 1993). The urea-N are degraded to NH_3-N which is less desirable because it is going to lead to inefficient use of N towards MP. It has been reported that some bacterial species prefer to use certain N sources (peptides, AA and NH_3-N) above others (Russell et al., 1992). Fermented forages contain large amounts of NPN because of protein degradation during fermentation (Broderick, 1996). Therefore, feeding diets that are high in

fermented forages can result in high ruminal NH_3-N concentrations which can lead to increased N excretion.

2.4.3. Fermentable Carbohydrate Supply

Urea-N recycling can be impacted by supplementation of ruminally fermentable carbohydrates as well as processing method of the carbohydrate source as previously described (Section 2.3.2). Synchronizing the amount of ruminal available N and energy have been thought to decrease the amount of N excreted (Castillo et al., 2000; Huntington and Archibeque, 2000). The theory behind nutrient synchrony was that more energy would be available when ruminal NH_3-N concentrations were high, in order to increase MP production and thereby reducing the amount of N excretion (Taweel et al., 2006). When diets are formulated to have nutrient synchrony there are a lot of variables that need to be factor in, like the rate of digestion, substrate amount available to a variety of ruminal microbes (Hall and Huntington, 2008), ability to predict N recycling to the GIT and N capture by the ruminal microbes (Cole and Todd, 2008).

When a non-structural carbohydrate source was supplemented at the same time as high quality fresh pasture was fed to cows, ruminal NH_3-N concentration decreased 22 to 43%, three to five hours after feeding (Kolver et al., 1998). This suggest that microbes could have utilized more NH_3-N but no difference in urine N excreted were found. When Kim et al. (1999) looked at the effect of infusing sucrose ruminally 6 h at each feeding (synchronous) vs 6 h after each feeding (asynchronous) on MP synthesis in cows consuming grass silage they found no effect on MP synthesis. A different pattern of ruminal NH_3-N concentration was reported between the synchronous treatment and the asynchronous diet but total ruminal NH_3-N concentrations did not differ. When lambs were fed a synchronous diet, a greater energy retention was found but it did not affect growth performance compared to when lambs were fed an asynchronous diet (Richardson et al., 2003). Horadagoda et al. (2008) demonstrated that when sheep were supplemented with slower degrading grains (sorghum, oats and barley) there was a synergistic effect on MP synthesis. When protein was supplemented on an asynchrony bases for not longer than 3 days apart there was no detrimental effect on growth and performance (Reynolds and Kristensen, 2008). These authors discuss the challenge to predict nutrient synchrony due to the lack of knowledge of all the factors that can regulate N recycling.

2.4.4. Frequency of N supply – Oscillating Dietary Crude Protein

Another approach to improving the efficiency of N utilization in ruminants is to feed oscillating dietary CP concentrations. The concept of feeding oscillating CP diets to ruminants evolved based on studies that investigated infrequent supplementation of ruminants with protein sources. For example, supplementing protein to sheep or cattle on low quality forages is a common practice. Producers attempt to decrease cost (labour and machinery) by only supplementing infrequently. Collins and Pritchard (1992) investigated the effect of supplementing SBM or corn gluten meal daily or every other day to lambs. The authors reported that treatment did not have an effect on ruminal SCFA, pH or total-tract DM digestibility; however, N retention increased two- to four-fold when CP supplements were provided every other day rather than daily to lambs. Brown et al. (1995) did not observe a difference in DM, ADF or NDF total-tract digestibility when sheep were supplemented with CP daily, every other day or every third day. Supplementation every other day tended to increase apparent total-tract N digestion (Brown et al., 1995). Beauty et al. (1994) observed that supplementing CP three times a week to steers fed wheat straw sustained high ruminal NH_3-N concentrations compared to daily supplementation. Since no adverse effects on animal performance were reported when animals were supplemented with a protein source every other day compared with daily, research has been conducted to investigate the effects of feeding oscillating CP diets to ruminants on productivity, N retention, and N excretion (Cole 1999; Ludden et al., 2002a,b, 2003; Cole et al., 2003; Archibeque et al., 2007a,b,c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011; Brown, 2014).

When an oscillating diet was fed to beef cattle (Cole et al., 2003; Archibeque et al., 2007a,b) and sheep (Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009) it had no effect on DMI compared to when a static diet was fed. In contrast, Doranalli et al. (2011) found a tendency for DMI to be lower with an oscillating CP dietary regimen (10.3% and 16.1% CP) was fed to sheep, compared with a static CP diet (12.7% CP). Average daily gain (ADG) increased by 30% when an oscillating CP (10.3 and 16.1% oscillated on a 48-h interval) diet was fed to sheep (Doranalli et al., 2011). In contrast, Ludden et al. (2003) noted no effect on ADG and feed efficiency when oscillating CP diets were used with steers fed low-quality roughage diets. Archibeque et al. (2007a) also noted no effect on ADG when oscillating dietary CP was used with steers on a finishing diet. There is limited research that investigated the effect of feeding

oscillating dietary CP levels on ruminal nutrient digestion and nutrient flow. Ludden et al. (2002a) reported no difference in apparent ruminal nutrient (OM, N, NDF and ADF) digestion, nutrient flow, nutrient disappearance in the small intestine and the hindgut when a high-forage oscillating diet was fed to sheep on a 48-h regimen compared to a static diet.

When the effect of feeding oscillating diets on SCFA production were investigated, it was reported that ruminal acetate (Ludden et al., 2002a; Doranalli et al., 2011), propionate (Doranalli et al., 2011), and total SCFA (Doranalli et al., 2011; Ludden et al., 2002a) concentrations were increased compared to when static diets were fed. Ruminal pH was unaffected when oscillating diets were fed to cows compared with static diets (Ludden et al., 2002a; Doranalli et al., 2011). A tendency for ruminal NH_3-N concentrations to be lower when high-forage diets were fed on an oscillating regimen to sheep compared to a static diet was reported (Ludden et al., 2002a). In contrast, no difference in ruminal NH_3-N concentration was observed when high-concentrate diets were fed on an oscillating regimen to sheep compared to a static diet (Doranalli et al., 2011). Plasma urea-N concentration was unaffected when oscillating diets were fed to animals compared to a static diet (Cole, 1999; Cole et al., 2003; Ludden et al., 2003; Archibeque et al., 2007b; Doranalli and Mutsvangwa 2009). Plasma urea-N is highly correlated with ruminal NH_3-N concentration (Hammond, 1983a; Hennessy and Nolan, 1988) and can be used as a parameter to monitor protein status in ruminants (Hammond, 1997).

When a high-forage oscillating diet was fed on a 48-h regimen (13.2 and 16.7% CP) compared to a static diet (15% CP), an increase in MP synthesis in sheep fed the oscillating dietary CP diet compared to those fed a static dietary CP diet was reported (Ludden et al., 2002b). In that study, MP production may have been limited since 65% bromegrass hay was incorporated in the diet, which might have restricted microbial growth due to limited availability of energy. Carbohydrate fermentation in the rumen and large intestine partially drives N recycling (Egan et al., 1986); thus, ruminants fed low-quality roughage diets would probably have less ruminal and post-ruminal fermentation than those fed high-concentrate diets (Owens et al., 1986), which may affect N recycling. There was a tendency for microbial NAN supply to the duodenum to be improved when the oscillating CP diet was fed to sheep compared to the static CP diet (Doranalli and Mutsvangwa, 2009). Similarly, Doranalli et al. (2011) reported that microbial NAN supply to the duodenum improved when an oscillating CP diet was fed to sheep compared to a static CP diet. The authors also reported that serosal-to-mucosal urea flux in using

chambers was 40% greater in the sheep fed the oscillating CP diet when compared to those fed the static diet which can imply that the higher microbial NAN supply to the duodenum is a result from higher urea-N recycling.

Archibeque et al. (2007b) compared the effect of feeding low (9.1%) and high (13.9%) oscillating CP finishing steer diets on a 48-h interval to feeding a static (11.8%) CP finishing steer diet and observed that the oscillating CP diet resulted in greater apparent total-tract N digestion. In contrast, is was reported that feeding an oscillating diet had no positive effects on apparent N digestion compared to a static CP diet (Cole 1999; Cole et al., 2003; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009). When comparing oscillating 48-h dietary CP regimens to static dietary CP diets, other studies found that total-tract digestibility of NDF and ADF (Doranalli and Mutsvangwa, 2009) were higher in animals fed the oscillating diet compared to those fed the static diet. In contrast, Ludden et al. (2002a,b) observed no difference when comparing oscillating 48-h dietary CP regimens to static dietary CP diet on apparent total-tract NDF and ADF digestibilities.

It has been reported that N intake was not affected when an oscillating diet was fed to beef cattle (Cole et al., 2003; Archibeque et al., 2007a,b) and sheep (Cole, 1999; Ludden et al., 2002a,b; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) compared to a static diet fed. Urinary (Cole 1999; Ludden et al., 2002b; Cole et al., 2003; Archibeque et al., 2007b, c) and fecal N (Ludden et al., 2002b; Cole et al., 2003; Archibeque et al., 2007c) outputs were unaffected when oscillating diets were fed to ruminants compared with static diets. In contrast, Archibeque et al. (2007b) and Doranalli et al. (2011) found that feeding an oscillating CP diet decreased fecal N output by 11-16% compared with feeding a static CP diet. Doranalli and Mutsvangwa (2009) and Doranalli et al. (2011) also found that by feeding a static diet urine N output increased up to 48% than when an oscillating diet was fed to animals. Studies in which a high-concentrate oscillating CP diet was fed reported that N retention can be increased by up to 68% in beef cattle (Cole et al., 2003; Archibeque et al., 2007b) and sheep (Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) when compared to feeding a static CP diet. Cole (1999) fed sheep 90% concentrated diets to evaluate the effects of feeding oscillating CP concentrations (10 and 15%) at 24- or 48-h intervals on N retention and observed that when compared to a static 12.5% CP diet, there was no effect on N retention when oscillating dietary CP diets were fed at 24-h intervals, but that N retention

increased by 38% when oscillating dietary CP diets were fed at 48-h intervals. Ludden et al. (2002) did not observe an improvement in N retention in lambs fed a high-forage oscillating diet (13% and 17% CP) on a 48-h interval compared to those fed a high-forage static diet (15% CP), and this could be because all diets were likely exceeding the supply of metabolizable protein and more N needed to be excreted. Thus, the decreased N excretion could indicate that there was greater urea-N recycling to the GIT and thereby more N was available for microbial synthesis leading to increased N retention and thereby improved production as well as improved environmental stewardship.

It was suggested by Cole (1999) that by feeding oscillating dietary CP concentrations, N recycling between the bloodstream and GIT particularly the rumen, might increase. Archibeque et al. (2007c) found evidence that an increased uptake of urea-N to the portal-drained viscera (PDV) might be part of the mechanism that increases N retention. In that study, the venous-arterial difference technique was used to investigate net flux of nitrogenous compounds across the PDV in growing wethers when an oscillating diet (9.5% and 15.5% CP) was fed compared with a static (12.5% CP) diet. Archibeque at al. (2007c) observed that there was a tendency for greater net flux of urea-N across the PDV in animals fed the oscillating dietary CP diet compared to those fed the static CP diet, even though N intakes were similar between diets. In further studies designed to elucidate if urea-N recycling to the rumen was a mechanism partly responsible for the increased efficiency of N utilization with oscillating CP diets, Doranalli et al. (2011) reported an increase in serosal-to-mucosal urea flux when sheep were fed the oscillating (10.3 and 16.1% CP) diets when compared to those fed the static diet (12.7% CP). Cole (1999) indicated that in order to see a benefit (N retention) when oscillating dietary CP diets are fed, it has to be in synchrony with retention time of digesta in the rumen. This would allow urea-N recycling rates to be greater when ruminal NH_3-N concentration were sub-optimal in terms of supporting microbial growth.

To my knowledge, there is only one study that has investigated the effects of feeding oscillating CP concentrations on N utilization in dairy cows (Brown, 2014). In that study, cows were fed a TMR with a 60:40 forage:concentrate ratio on a 48-h oscillating regimen (10.3 and 16.4% CP) or a static CP diet (13.4% CP). There was no dietary effect on DMI, N intake, milk yield, milk composition, urinary N and fecal N (expressed as a % of N intake). On the second day of feeding the 10.3% CP diet, milk yield and MUN decreased. Brown (2014) used an

oscillation frequency of 48-h for dairy cows which, presumably, was based on the published studies with finishing cattle and sheep. Rumen digesta retention time can be influenced by a variety of factors, such as dietary forage:concentrate ratio and level of DMI. Normally, dairy cows are at higher levels of DMI and are fed higher forage diets (around 50% of dietary DM) compared to finishing cattle (around 80-90% of dietary DM), so it is plausible that the oscillation frequencies that have been used in research with finishing cattle might not be suitable for dairy cows due to differences in rumen retention time of digesta. Therefore, it is important to determine if the 48-h oscillation regimen that has typically been used in research studies with beef cattle and sheep is suitable for use in dairy cows.

The dietary CP levels that were used in the study by Brown (2014) (i.e., 10.3 and 16.4% CP for the oscillating treatment, and 13.4% CP for the static treatment) appear to be rather low for high-producing dairy cows (particularly the 10.3 and 13.4% CP diets) based on NRC (2001) recommendations. Therefore, it is unknown how dairy cows would respond to feeding oscillating dietary CP concentrations when values are closer to NRC (2001) recommendations for high-producing dairy cows. Brown (2014) also determined the effects of feeding oscillating dietary CP concentrations on N utilization. In that regard, spot urine samples were collected and then urinary excretion was estimated using creatinine as a urine marker (Valadares et al., 1999), with urinary N excretion being calculated based on equations from Kauffman and St-Pierre (2001). Fecal N excretion were also estimated based on an equation as follows: fecal N (g/d) = N intake (g/d) – (milk N (g/d) + urine N (g/d)). Because N urine and fecal excretions were only estimated and not based on total collections of feces and urine, the data on N balance reported by Brown (2014) might not be as reliable as data that would be obtained using total collections. Also, Brown (2014) did not investigate the effects of feeding oscillating diets to dairy cows on rumen N utilization and MP synthesis.

2.5 Summary

There is a need to increase food production with the increasing population which leads to more intensive farming practices. Intensive livestock systems can cause accumulation of nutrients resulting in environmental pollution. Ruminants are not very efficient in utilizing N, which results in excessive N excretion into the environment. Excess N in the environment leads to contamination of ground water, eutrophication of surface water, smog and acidification of

cropland (Galloway, 2002; Dijkstra et al., 2011). A mitigation strategy to increase N utilization in ruminants is to feed diets on an oscillating CP regimen. It has been reported that by feeding oscillating CP diets to sheep (Cole, 1999; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) and steers (Cole et al., 2003; Archibeque et al., 2007b), N utilization efficiency can be improved. To my knowledge, only one study investigated the effect of feeding oscillating CP diets to dairy cows (Brown, 2014). When an oscillating diet was fed on a 48-h basis to dairy cows no effects on DMI, N intake, milk yield and milk composition, and excretion of urinary N and fecal N (expressed as a % of N intake) compared to feeding a static CP diet were reported (Brown, 2014).

2.6 Hypothesis

Feeding oscillating CP diets on a 48-h regimen to dairy cows will improve the efficiency of N utilization (i.e., greater N retention and milk N secretion) compared to feeding static or oscillating CP diets on 24- or 72-h regimen.

2.7 Objectives

To determine the optimum frequency of feeding oscillating dietary CP (i.e., 24-, 48- or 72-h basis) in dairy cows;

To determine the effects of feeding oscillating CP diets on ruminal fermentation characteristics (NH_3, SCFA and pH), microbial protein synthesis, ruminal outflow of nutrients, and N balance in dairy cows; and

To determine the effects of feeding oscillating CP diets on feed intake, milk production and milk composition in dairy cows.

3. MATERIALS AND METHODS

The study was conducted in the Metabolism Wing at the Rayner Dairy Research and Teaching Facility (University of Saskatchewan). Experimental cows were cared for and handled in accordance with the Canadian Council of Animal Care regulations and the University of Saskatchewan Animal Care Committee approved all experimental procedures that were conducted in the animals (UCACS Protocol No. 20040048).

3.1 Animals, Experimental Design, and Dietary Treatments

Eight Holstein lactating dairy cows (714 ± 36 kg BW; 114 ± 15 DIM at the beginning of the experiment) were housed individually in tie-stalls and used in a replicated 4 × 4 Latin square design experiment with 30-d periods (consisting of 18 d of dietary adaptation and 12 d of sample and data collection). To facilitate ruminal measurements and omasal digesta sampling, one Latin square contained four ruminally-cannulated cows. Treatments were a diet containing 16.5% CP (designated STATIC) fed on a continuous basis, and diets containing 13.5% and 19.5% CP that were fed on an oscillating basis at 24 (OSC-24), 48 (OSC-48), or 72 (OSC-72) h (Table 3.1). The STATIC (16.5% CP) diet was formulated to meet the metabolizable protein requirements of dairy cows (NRC, 2001), whereas the 13.5% and 19.5% CP diets were formulated to under- or over-supply metabolizable protein, respectively. Cows were fed experimental diets as total mixed rations (TMR), which comprised of a 51:49 forage:concentrate ratio (on a DM basis). Animals were fed daily at 0900 and 1700 h for ad libitum intake.

3.2 Data and Sample Collection

For each experimental period, feeds offered and orts were recorded daily over the last 12 d (d 19 to 30). Samples of the TMR and orts were collected daily on d 19 to 24 and stored at -20ºC until analysis. Cows were milked three times daily at 0430, 1300, and 1900 h, and milk weights were recorded daily (d 19 to 30). During each period, milk samples were collected from all three milkings on d 19 to 22 for the STATIC, OSC-24 and OSC-48 treatments, and on d 19 to 24 for the OSC-72 treatment. The milk sampling schedule was designed to encompass at least one oscillation cycle for each of the oscillating treatments. A preservative (2-bromo-2-nitropropane-1-2-diol) was added to the milk samples at collection. The milk samples were

Table 3.1 Feed ingredient and chemical composition of diets fed to dairy cows.

	Diet		
Ingredient Composition, % of diet DM	13.5 % CP	16.5 % CP	19.5 % CP
Barley silage	29.6	29.7	29.6
Alfalfa hay	21.5	21.5	21.5
Rolled barley grain	24.00	21.50	17.60
Ground corn grain	10.80	8.26	4.30
Canola meal	3.58	4.67	7.89
Soybean meal	0.72	3.95	7.17
Pea and lentil grain	3.58	3.59	3.58
Corn gluten meal	0.36	0.93	2.51
Molasse cane	0.56	0.56	0.56
Corn distillers	1.56	1.57	1.56
Palmitic acid	0.72	0.72	0.72
Vitamin and mineral premix[1]	3.78	3.78	3.78
Chemical composition			
DM, %	60.7	60.7	60.7
CP, % of DM	14.3	17.1	20.3
NDF, % of DM	30.4	30.5	30.9
ADF, % of DM	19.4	19.5	19.9
Starch[2], % of DM	29.0	25.3	20.4
EE, % of DM	3.93	3.92	3.92
NE_L[3], Mcal/kg of DM	1.61	1.61	1.61

[1] Contained (/kg of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16%, Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.
[2]Estimated from feed ingredient starch content based on CPM Dairy Ration Analyzer v3.0.0.
[3]Calculated from CPM Dairy Ration Analyzer v3.0.0.

pooled by day proportionally based on milk yield and then submitted to CanWest DHI Laboratory (Edmonton, AB) for analysis of CP, fat, lactose, and milk urea-nitrogen (MUN) using a near infrared analyzer (Foss System 4000, Foss Electric, Hillerod, Denmark) according to AOAC (1990).

The four ruminally-cannulated cows were used to delineate dietary effects on ruminal fermentation, MP production, omasal nutrient flow, total-tract nutrient digestion, and N balance. The omasal sampling technique was used to obtain samples of digesta leaving the rumen to determine the site and extent of nutrient digestion while three markers were infused continuously (d 13 to 24). To quantify omasal digesta flow, indigestible NDF (iNDF; Reynal et al., 2005), $YbCl_3$ (Siddons et al., 1985), and Cr-EDTA (Udén et al., 1980) were used as digesta markers for the large particle (LPP), small particle (SPP), and fluid (FPP) phases, respectively.

A microbial marker, $[^{15}NH_4]_2 SO_4$, (10 atom percent excess ^{15}N; Cambridge Isotope Laboratories, Andover, MA), was used to quantify ruminal MP production as described by Reynal et al. (2005). A 500-mL ruminal digesta sample was collected to determine background ^{15}N abundance and marker concentrations prior to the initiation of infusion of marker solutions on d 13. After the background sample was taken, a priming dose equivalent to half the daily dose of the marker solutions ($YbCl_3$, $[^{15}NH_4]_2 SO_4$, and Cr-EDTA) was administered on d 13. Marker solutions were then continually infused into the rumen using a peristaltic pump (Model: 205U, Watson and Marlow, Cornwall, UK) for 10 d (d 13 to d 23) for the STATIC, OSC-24 and OSC-48 treatments and 12 d (d 13 to d 25) for the OSC-72 treatment. Marker solutions were infused at a constant rate of 1 L/d, (providing 2.77 g of Cr, 3.35 g of Yb, and 0.22 g of ^{15}N per day). The weights of marker solutions infused were recorded daily. For analysis of Cr and Yb concentrations in the marker solutions, a 50-mL sub-sample of marker solution was collected during each experimental period and stored at room temperature.

Sampling of ruminal contents and omasal digesta began at 0900 h on day 19. For the STATIC, OSC-24 and OSC-48 treatments, sampling was conducted at 0900, 1700 and 0100 h on d 19, 1300, 2100 and 0500 h on d 20, 1300, 2100 and 0500 h on d 21, and 0900, 1700 and 0100 h on d 22. For the OSC-72 treatment, sampling was conducted at 0900, 1700 and 0100 h on d 19, 1200, 2000 and 0400 h on d 20, 1500, 2300 and 0700 h on d 21, 0900, 1700 and 0100 h on d 22, 1200, 2000 and 0400 h on d 23, and 1500, 2300 and 0700 h on d 24.

For all treatments, the collected samples were representative of a 24-h feeding cycle, and the sampling protocols ensured that an equal number of representative samples were collected when cows on the oscillating dietary CP diets were receiving the 13.5% and 19.5% CP diets. In order to collect omasal digesta, the sampling technique described by Huhtanen et al. (1997) was used. To minimize potential disturbance of normal digesta passage and function, the omasal canal was located by hand and a sampling tube was inserted through the ruminal cannula at each sampling time. A 425-mL sample of omasal digesta was collected through the tube and divided into 100-, 125- and 200-mL subsamples at each sampling. The 100- and 200-mL subsamples were pooled by cow over the sampling period to yield 1.2- and 2.4-L composite samples for the STATIC, OSC-24 and OSC-48 treatments, and 1.8- and 3.6-L composite samples for the OSC-72 treatment, respectively. Omasal digesta samples were stored at -20ºC for later analysis. The 125-mL sub-samples were placed immediately in an ice-bath and pooled over 2 sampling times in order to yield a 250-mL composite sample. The 250-mL composite sample was used to isolate particle-associated (PAB) and fluid-associated (FAB) bacteria using filtration and differential centrifugation as described by Brito et al. (2009). Briefly, the 250-mL composite samples were filtered through two layers of cheesecloth, the solids were then washed with 250 mL of 0.85% (wt/vol) NaCl solution and then filtered through the two layers of cheesecloth. The retained solids and 175 mL of a chilled 0.85% (wt/vol) NaCl solution with 0.1% (wt/vol) Tween-80 were then placed in a 500-mL PAB container, were mixed thoroughly and stored in an ice bath until further analysis. The fluid obtained from the first step was centrifuged (1,000 × *g* at 5°C for 5 min) which resulted in a pellet which was then placed into the PAB container. The PAB contents were blended for 20 s and stored at 5°C for 24-h. The fluid obtained after the pellet was removed was then again centrifuged (11,300 × *g* at 5°C for 30 min). After centrifugation, the fluid was discarded and the pellet was re-suspended in 50 mL of McDougall's buffer (McDougall, 1948) and re-centrifuged at 11,300 × *g* at 5°C for 30 min. The pellet obtained from this step was then stored at -20ºC for FAB analyses. After 24-h, the PAB content were filtered through two layers of cheesecloth. The fluid was then processed as described previously for FAB except that, after the initial centrifugation (1,000 × *g*, 5°C, 5 min), the pellet obtained was discarded. The pellet obtained from this step was then stored at -20ºC for PAB analyses. All pellets were pooled by cow by period.

At the same time, points as for omasal sampling, approximately 1,000 mL (250 mL/region) of ruminal contents were collected from the cranial-ventral, caudal-ventral, ventral, and cranial-dorsal rumen for the determination of ruminal fermentation characteristics. The 1,000 mL ruminal contents were filtered through 4 layers of cheesecloth. Ruminal fluid pH was then measured immediately using a portable pH meter (VWR Symphony Model SP70P). For the determination of ruminal SCFA and NH_3 concentrations, two 10-mL sub-samples of ruminal fluid were collected and mixed with chilled 25% (wt/vol.) meta-phosphoric acid (H_2PO_4) or 1% H_2SO_4, respectively, and stored at -20°C for later analysis.

In order to determine apparent total-tract nutrient digestibility and N balance, total urine and feces collection were conducted as described by Plaizier et al. (2000). For the STATIC, OSC-24 and OSC-48 treatments, total urine and fecal collections were conducted for 4 d (d 25 to 28), whereas collections for OSC-72 were for 6 d (d 25 to 30). These sampling protocols were designed to encompass at least one oscillation cycle for the oscillating treatments. Large steel trays were positioned over the gutter behind each tie-stall in order to collect feces. Twice daily, fecal output for each cow was transferred into a pre-weighed plastic container, weighed and the weight recorded. After the feces were mixed thoroughly, a 2.5% sub-sample was taken daily and pooled for each cow during each collection period and stored at -20˚C pending chemical analysis. Collection of urine was conducted using indwelling bladder catheters (Bardex Foley bladder catheters, 26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) as described by Crutchfield (1968). Bladder catheters were inserted 24-h prior to the initiation of urine collection (at 0900 h on day 24), but bladder catheters were not connected to the urine collection tubing until d 25 at 0900 h. Starting on d 25, urine was collected into pre-weighed 20-L carboy polyethylene containers which contained 150 mL of concentrated HCl to acidify the urine and, thereby, prevent bacterial growth and the loss of volatile NH_3-N. Total urine output was recorded daily and a 5% sub-sample of the daily output was taken after the urine was mixed thoroughly. Daily urine samples were pooled for each cow during each collection period and stored at -20˚C until analyzed for total N. A 2-mL subsample of urine was taken daily, diluted with 8 mL of distilled water and stored at -20°C for later determination of urea-N.

Blood samples were collected from the tail vein on d 26 and d 27 for the STATIC and OSC-24 treatments, on d 26 and 28 for the OSC-48 treatment, and on d 27 and d 30 for the OSC-

72 treatment before the morning feeding. Blood samples were centrifuged (1,500 x g, 4°C, 15 min) and the plasma obtained was stored at -20°C for later analysis of urea-N. Using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA), ruminal pH was continuously measured every 30 sec for 4 d (d 25 to 28) for the STATIC, OSC-24 and OSC-48 treatments, and 6 d (d 25 to 30) for the OSC-72 treatment as described by Penner et al. (2006).

3.3 Sample Analyses

At the end of the trial, frozen TMR, orts and fecal samples were thawed overnight at room temperature and then dried in a forced-air oven at 60°C for 48 h to determine DM (AOAC, 1990; method 930.15). Dried TMR, orts, and feces were ground through a 1-mm screen (Christy and Norris Ltd., Chelmsford, England). Ground TMR, orts and fecal samples were pooled per cow for each experimental period and analyzed for organic matter (OM; AOAC, 2000; method 942.05), CP (AOAC, 2000; method 990.03; Leco FP-528 Nitrogen Combustion Analyzer), ether extract (AOAC, 2000; method 920.39), acid detergent fibre (ADF; AOAC, 2000; method 973.18), and neutral detergent fibre (NDF; Van Soest et al., 1991). Heat-stable α-amylase and sodium sulfite were used for NDF determination.

Omasal digesta samples (2.4- and 3.6-L composite samples) were thawed at room temperature. The samples were then separated into large particle (LPP), small particle (SPP), and fluid (FPP) omasal digesta phases (Brito et al. 2009). Briefly, the omasal digesta was filtered through one layer of cheesecloth and the solids obtained from the cheesecloth were defined as LPP. The filtrate was then centrifuged at 1000 × g for 5 min at 5°C. The fluid and pellet obtained was defined as FPP and SPP, respectively. The LPP, SPP and FPP samples were then freeze-dried, after which the dried samples were ground through a 1-mm screen (Christy and Norris Ltd., Chelmsford, England). A 1-g sample of each phase (LPP, SPP and FPP) was ashed for 8 h at 550°C in a muffle furnace (AOAC, 1990), followed by nitric acid digestion (Vicente et al. 2004). Atomic absorption spectrophotometry (Perkin-Elmer 2300, Perkin-Elmer Corp, Norwalk, CT) and atomic emission spectroscopy (Varian Spectra 220, Varian, Mulgrave, Australia) were used to determine Cr and Yb concentrations, respectively. Also, 12-day ruminal in situ incubations were used to determine the concentrations of iNDF in the duplicate TMR, orts and LPP samples, and triplicate SPP samples (Ahvenjärvi et al., 2000). Briefly, 1-g samples of TMR,

orts, LPP, and SPP were weighed into 5- × 10-cm nylon mesh bags (6 μm pore size; part no. 03-6/5, Sefar America Inc., Depew, NY) and then incubated in the rumen of a ruminally-cannulated cow for 12 d. Immediately after being removed from the rumen, the bags were rinsed in water for 30 min and then analysed for NDF as previously described.

Due to marker dysfunction, the triple-maker method could not be used to reconstitute the omasal true digesta (OTD). The spare whole omasal digesta (WOD) samples (1.2- and 1.8-L composite samples) were freeze-dried. Concentration of Cr in the WOD was measured by using the methods described above, and then used to calculate omasal DM flow. Concentrations of OM, CP, ether extract, ADF and NDF in WOD samples were determined using procedures already described above. For the analysis of NH_3-N in the WOD samples, 10 mL Na-citrate buffer (0.07 M; pH 2.2) were added to 0.5 g WOD sample, vortexed and then held in a forced-air oven at 39°C for 30 min. The samples were then centrifuged (18,000 × g, 4°C, 15 min) and the fluid were then analyzed for NH_3-N using a phenol-hypochlorite assay (Broderick and Kang 1980).

The ^{15}N background (500 mL) samples were freeze-dried, ground through a 1-mm screen (Christy and Norris Ltd., Chelmsford, England) and pulverized with a ball mill before analysis of non-ammonia nitrogen (NAN) and ^{15}N enrichment. A mortar and pestle were used to ground the FAB and PAB pellets after freeze-drying. To determine ^{15}N enrichment in the samples (^{15}N background, FAB, PAB and WOD), the samples were prepared according to Brito et al. (2009). Briefly, samples containing approximately 100 μg of N were weighed into tin capsules (5- × 8-mm; Elemental Microanalysis Limited, Okehampton, UK), then 50 μL K_2CO_3 (72 mM) was added to each capsule before drying in a forced-air oven at 60°C for 24-h). Finally, an elemental analyzer and continuous flow isotope ratio-mass spectrometry were used to measure ^{15}N enrichment by combustion of the samples to nitrogen gas.

Frozen ruminal fluid sub-samples that were preserved with 25% H_2PO_4 were thawed at room temperature and then centrifuged at 18,000 x g for 15 min at 4°C. A 0.9-mL sub-sample of filtered ruminal fluid was added to a clean, dry vial and an internal standard (crotonic acid, 1 mg/mL) was then added to each vial. Ruminal SCFA were then separated and quantified by using gas chromatography (Erwin et al. 1961). Frozen ruminal fluid sub-samples that were preserved with 1% H_2SO_4 were thawed at room temperature, centrifuged (18,000 x g, 4°C, 10 min) and then used for ruminal NH_3-N as described by Broderick and Kang (1980).

Plasma and urinary urea-N was determined based on the phenol-hypochlorite assay method of Fawcett and Scott (1960). Pooled urine samples were used to determine total N by using the macro-Kjeldahl procedure (AOAC, 1990).

3.4 Calculations

The pH that was measured every 30s were averaged each minute for each day. The minimum pH, mean pH and maximum pH were then reported from the summarized data. Ruminal acidosis occurrence and severity were determined from the pH data using two pH thresholds (5.8 and 5.5; Penner et al., 2007). When ruminal pH was below the first threshold (5.8), it was considered that ruminal acidosis occurred. When ruminal pH was between the two thresholds (5.8 and 5.5), the ruminal acidosis were considered to be mild. When ruminal pH was below the second threshold (5.5), the ruminal acidosis was considered to be severe (Penner et al., 2007). Calculations were done in order to determine the duration span (min/d) and aggregated area (pH x min) that the ruminal pH was underneath every threshold (Penner et al., 2007). Calculations of bacterial N yield using ^{15}N as a microbial marker were conducted as described by Brito et al. (2009). Briefly, omasal flow of NAN was calculated as the difference between total omasal N flow and NH_3-N flow. In the 16 background ^{15}N digesta samples, the mean natural enrichment of ^{15}N was 0.36833. In the WOD and bacterial samples, ^{15}N enrichment (APE) was calculated as follows: ^{15}N APE = ^{15}N atom % in sample - ^{15}N atom % in background sample. The omasal flow of total bacterial NAN was calculated as total WOD NAN flow × (WOD ^{15}N APE ÷ bacterial ^{15}N APE). Omasal flow of NANMN (non-NH_3, microbial N) was calculated as total NAN flow – total bacterial NAN flow.

3.5 Statistical Analysis

In Period 2, a ruminally-canulated cow fed the OSC-72 diet had mastitis, which was unrelated to the dietary treatment. For that cow, data on DMI, milk production and composition, ruminal fermentation characteristics (NH_3, SCFA and pH), microbial protein synthesis, and ruminal outflow of nutrients were excluded from the statistical analysis. Feed intake and milk yield and composition data were analyzed as a replicated 4 x 4 Latin square using the Proc Mixed procedure of SAS (9.4, SAS Institute Inc., Cary, NC). The following model was used: $Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl}$ (Y_{ijkl} = dependent variable, μ = overall mean, S_i = fixed effect of square i, P_j = fixed effect of period j, $C_{k(i)}$ = random effect of cow k (within square

i), T_l = fixed effect of dietary treatment l, ST_{il} = interaction between square i and treatment l, and E_{ijkl} = residual error). Ruminal pH, ruminal concentrations of SCFA and NH_3-N, plasma urea-N, N balance, MP production, and omasal nutrient flow data were analyzed as a 4 x 4 Latin square using the Proc Mixed procedure of SAS. The following model were used: $Y_{ijk} = \mu + P_i + C_j + T_k + E_{ijk}$ (Y_{ijk} = dependent variable, μ = overall mean, P_i = fixed effect of period i, C_j = random effect of cow j, T_k = fixed effect of dietary treatment k, and E_{ijk} = residual error). Orthogonal contrasts were used to test for contrast between the following treatments: STATIC vs OSC diets, OSC-24 vs OSC-48 and OSC-48 vs OSC-72. Treatment effects were declared significant when $P \leq 0.05$, and tendencies when $0.05 < P \leq 0.10$.

4. Results

4.1 Dietary Characteristics

The dietary ingredients and chemical compositions of experimental TMR fed to lactating dairy cows are presented in Table 3.1 The actual CP concentrations were 17.1% for the STATIC, and 14.3 and 20.3% for the oscillating CP diets, which deviated from the target CP concentrations due to large variations in forage CP content.

4.2 Feed Intake and Milk Production and Composition

The results presented in Table 3.2 on feed intake and milk production are from all 8 cows that were used in the study. Dry matter intake (mean = 26.6 kg/d) and milk production (mean = 36.4 kg/d) were not affected ($P \geq 0.19$) by dietary treatment. Milk protein yield was greater (P = 0.03) in cows fed the STATIC diet as compared to those fed the OSC diets (1.22 vs. 1.14 kg/d). Milk urea-N tended (P = 0.10) to be greater (by 8%) in cows fed the OSC-48 diet compare to those fed the OSC-24 diet. Plasma urea-N concentration was unaffected ($P \geq 0.31$) by dietary treatment.

Table 3.2. The influence of feeding oscillating dietary CP concentrations on dry matter intake (DMI) and milk yield and composition in dairy cows[1].

	Treatments					Contrast, *P* value		
Item	STATIC	OSC-24	OSC-48	OSC-72	SEM	STATIC vs. OSC	OSC-24 vs. OSC-48	OSC-48 vs. OSC-72
DM intake, kg/d	26.7	26.8	26.5	26.6	0.85	0.95	0.74	0.88
Milk yield, kg/d	37.6	35.7	36.4	36.1	1.38	0.19	0.63	0.86
Milk fat, %	3.55	3.58	3.59	3.62	0.14	0.56	0.88	0.78
Milk fat yield, kg/d	1.33	1.25	1.32	1.31	0.05	0.45	0.18	0.77
Milk protein, %	3.23	3.21	3.12	3.17	0.09	0.34	0.30	0.63
Milk protein yield, kg/d	1.22	1.12	1.15	1.15	0.04	0.03	0.52	0.92
Milk lactose, %	4.37	4.37	4.39	4.39	0.10	0.81	0.51	0.99
Milk lactose yield, kg/d	1.55	1.53	1.66	1.59	0.06	0.55	0.17	0.49
Milk urea-N, mg/dL	15.4	15.2	16.4	16.1	0.63	0.38	0.10	0.71
Feed efficiency[2]	1.42	1.34	1.37	1.36	0.06	0.18	0.53	0.86
Plasma urea-N, mg/dL	20.0	18.4	20.2	20.7	1.43	0.89	0.31	0.74

[1]Values are least squares means obtained from 8 cows.

[2]Feed efficiency = milk yield/DMI.

4.3 Ruminal Fermentation Characteristics

Dietary treatments fed to cows had no effect ($P \geq 0.15$) on daily mean, minimum and maximum ruminal pH, the duration span (min/d) and aggregated area (pH x min) that the ruminal pH was beneath every threshold (5.5 and 5.8; Table 3.3). Ruminal SCFA concentrations were largely unaffected by dietary treatment ($P \geq 0.25$), except that cows fed the OSC-48 diet tended to have a lower ($P = 0.09$) ruminal concentration of propionate compared to cows fed the OSC-72 diet (Table 3.4). Ruminal SCFA concentrations over time were largely unaffected by dietary treatment (Appendix Figure 1-4). Cows fed the OSC diets tended to have greater ($P = 0.05$) ruminal NH_3-N concentrations compared to those fed the STATIC diet (12.7 vs. 11.6 mg/dL). In addition, cows fed the OSC-48 diet had a greater ruminal NH_3-N concentration compared to those fed the OSC-24 ($P = 0.05$) and the OSC-72 ($P < 0.01$) diets. Cows fed the STATIC, OSC-24 and OSC-48 diets did not have ruminal NH_3-N concentrations below 5.0 mg/dL (Appendix Figure 5a) whereas, cows fed the OSC-72 diet had ruminal NH_3-N concentrations below 5.0 mg/dL on d 5 at 0400, d 6 at 1500 and 2300 (Appendix Figure 5b).

4.4 Nitrogen Balance and Apparent Total-tract Digestibility

Nitrogen intake and urinary, fecal and milk N outputs were unaffected by dietary treatments fed to cows ($P \geq 0.39$; Table 3.5); however, total N excretion as a percentage of N intake tended ($P = 0.10$) to be greater in cows fed the OSC-24 diet compared to those fed the OSC-48 diet. Apparent N balance was greater ($P = 0.03$; by 22.5%) in cows fed the OSC diets compared to those fed the STATIC diet (195.3 vs 159.4 g/d). Cows fed the OSC-48 diet had a higher ($P < 0.01$, by 41%) apparent N balance compared to those fed the OSC-24 diet. No differences in apparent total-tract digestibilities were observed due to dietary treatment ($P \geq 0.19$; Table 3.6).

Table 3.3. The influence of feeding oscillating dietary CP concentrations on ruminal pH in dairy cows[1].

	Treatments					Contrast, *P* value		
Item	STATIC	OSC-24	OSC-48	OSC-72	SEM	STATIC vs. OSC	OSC-24 vs. OSC-48	OSC-48 vs. OSC-72
Ruminal pH								
Mean	6.22	6.18	6.10	6.13	0.07	0.34	0.49	0.80
Minimum	5.72	5.64	5.51	5.58	0.07	0.15	0.28	0.51
Maximum	6.76	6.78	6.64	6.65	0.05	0.34	0.16	0.92
Duration, min/d								
pH < 5.8	90.4	147	241	136.7	81.5	0.40	0.45	0.40
pH < 5.5	3.56	38.4	29.6	12.8	21.3	0.33	0.76	0.56
Area, pH × min/d								
pH < 5.8	9.23	28.8	37.5	19.8	15.9	0.30	0.69	0.43
pH < 5.5	0.09	4.56	3.58	1.18	2.66	0.29	0.77	0.48

[1]Values are least squares means obtained from 4 cows.

Table 3.4. The influence of feeding oscillating dietary CP concentrations on ruminal fermentation characteristics in dairy cows[1].

	Treatments					Contrast, *P* value		
Item	STATIC	OSC-24	OSC-48	OSC-72	SEM	STATIC vs. OSC	OSC-24 vs. OSC-48	OSC-48 vs. OSC-72
Ruminal SCFA, m*M*								
Acetate	82.0	78.8	78.9	79.6	2.79	0.25	0.98	0.83
Propionate	27.0	26.5	25.2	27.6	1.07	0.52	0.24	0.09
Butyrate	15.8	15.5	16.3	15.4	1.02	0.94	0.51	0.48
Isobutyrate	1.03	1.01	1.11	0.99	0.05	0.88	0.13	0.11
Valerate	1.79	1.79	1.79	1.76	0.08	0.88	0.97	0.63
Isovalerate	1.65	1.60	1.68	1.46	0.10	0.49	0.52	0.15
Total SCFA	129.3	125.3	125.0	126.7	4.20	0.26	0.94	0.69
Acetate:propionate ratio	3.08	2.98	3.15	2.94	0.13	0.70	0.35	0.32
Ruminal NH_3-N, mg/dL	11.6	12.6	13.9	11.5	0.86	0.05	0.05	<0.01

[1]Values are least squares means obtained from 4 cows.

Table 3.5. The influence of feeding oscillating dietary CP concentrations on urinary N excretion, fecal excretion and apparent N balance in dairy cows[1].

	Diet					Contrast, *P* value		
Item	STATIC	OSC-24	OSC-48	OSC-72	SEM	STATIC vs. OSC	OSC-24 vs. OSC-48	OSC-48 vs. OSC-72
N intake, g/d	760.22	750.4	779.0	763.3	29.7	0.78	0.14	0.39
Urinary excretion								
Total, kg/d	33.3	33.0	33.0	33.0	3.06	0.89	0.98	0.98
Total N, g/d	207	208	186	184	14.2	0.39	0.28	0.94
Total N, % N intake	27.2	27.7	24.1	24.2	1.83	0.39	0.19	0.95
Urea-N, g/d	130.8	124.1	135.7	122.8	6.18	0.69	0.28	0.23
Urea-N, % of urinary N	65.3	60.7	74.4	66.9	5.62	0.77	0.14	0.38
Fecal excretion								
DM, kg/d	7.80	7.98	7.73	7.94	0.34	0.81	0.53	0.62
N, g/d	205	209	191	208	11.1	0.85	0.20	0.21
N, % N intake	28.2	22.5	19.0	26.8	3.12	0.21	0.48	0.14
Total N excretion								
g/d	410.2	413.5	380.6	393.3	21.6	0.51	0.23	0.63
% N intake	54.7	55.1	48.6	51.3	2.15	0.29	0.10	0.42
Milk N, g/d	182.2	174.2	179.8	194.4	9.06	0.94	0.59	0.29
Apparent N balance, g/d	159.4	158.4	224.0	203.6	14.5	0.03	<0.01	0.31

[1]Values are least squares means obtained from 4 ruminally-cannulated cows.

Table 3.6. The influence of feeding oscillating dietary CP concentrations on apparent total-tract digestibilities in dairy cows[1].

	Diet					Contrast, *P* value		
Item	STATIC	OSC-24	OSC-48	OSC-72	SEM	STATIC vs. OSC	OSC-24 vs. OSC-48	OSC-48 vs. OSC-72
Apparent total-tract digestibility, %								
DM	70.7	69.5	71.4	70.1	0.94	0.73	0.24	0.40
OM	72.5	71.2	73.0	71.7	0.80	0.60	0.19	0.34
CP	73.2	72.7	74.9	72.7	1.10	0.88	0.19	0.18
NDF	47.6	46.6	49.5	47.6	1.91	0.89	0.31	0.50
ADF	43.4	39.2	43.5	40.6	2.07	0.30	0.14	0.28
EE	84.0	83.5	86.4	87.8	1.07	0.19	0.12	0.42

[1]Values are least squares means obtained from 4 ruminally-cannulated cows.

4.5 Ruminal Digestion and Nutrient Flow

There was no diet effect ($P > 0.05$) on DMI for the 4 ruminally-cannulated cows (Table 3.7). Cows fed the STATIC diet tended to have greater omasal DM ($P = 0.10$) and OM ($P = 0.09$) flows compared to cows fed the OSC diets. Dry matter and NDF apparent ruminal digestion were lower ($P < 0.05$) for cows fed the STATIC diet compared to the cows fed the OSC diets. Dry matter apparent ruminal digestion as a percentage of DMI were lower ($P = 0.05$) for the cows fed STATIC diet compared to those fed the OSC diets. Organic matter intake was higher in the cows fed the OSC-72 diet compared to the cows fed the OSC-48 diet ($P = 0.05$; by 20%). Organic matter apparent digested in the rumen was lower ($P = 0.05$) in the STATIC diet compared to the OSC diets. Additionally, there was no diet effect ($P = 0.44$) on the amount and percentage of ADF digested in the rumen.

4.6 Omasal N Fraction Flow and Microbial Protein Synthesis

There was no diet effect ($P > 0.05$) on N intake for the 4 ruminally-cannulated cows (Table 3.8). Nitrogen apparent and true digested in the rumen were unaffected by diet ($P \geq 0.15$). Nitrogen flow at omasal canal tended ($P = 0.06$) to be greater in cows fed the STATIC diet compared to those fed the OSC diets. Non-ammonia-nitrogen tended to be higher ($P = 0.06$) for cows fed the STATIC diet compared to the cows fed the OSC diets Non-ammonia-non-bacterial-nitrogen as a % of DMI were higher for cows fed the STATIC diet compared to the cows fed the OSC diets (0.789 vs 0.637 g/d). Non-ammonia-non-bacterial-nitrogen tended to be greater ($P = 0.06$; by 22%) in the cows fed the STATIC diet compared to the cows fed the OSC diets. Non-ammonia-non-bacterial-nitrogen as a % of DMI were higher ($P = 0.04$) for cows fed the STATIC diet compared to the cows fed the OSC diets (0.789 vs 0.637 g/d). Total bacterial NAN flow as a % of NAN flow tended ($P = 0.10$) to be higher in cows fed the OSC-48 compared with cows fed the OSC-72 diet.

Table 3.7. The influence of feeding oscillating dietary CP concentrations on nutrient flow and ruminal digestion in dairy cows[1].

	Treatments					Contrast, *P* value		
Item	STATIC	OSC-24	OSC-48	OSC-72	SEM	STATIC vs. OSC	OSC-24 vs. OSC-48	OSC-48 vs. OSC-72
DM								
Intake, kg/d	26.5	25.8	27.1	27.9	0.86	0.53	0.12	0.39
Omasal flow, kg/d	19.9	17.3	17.7	17.7	1.69	0.10	0.82	1.00
Apparent digestion, kg/d	6.68	8.48	9.45	10.2	1.29	0.04	0.42	0.61
Apparent digestion, % of DMI	25.3	33.3	34.9	36.7	5.31	0.05	0.73	0.74
OM								
Intake, kg/d	24.4	23.8	24.1	25.3	0.32	0.94	0.52	0.05
Omasal flow, kg/d	15.2	13.3	13.4	13.3	1.34	0.09	0.88	0.93
Apparent digestion, kg/d	9.19	10.6	10.7	11.9	1.25	0.09	0.89	0.38
Apparent digestion, % of OM intake	37.6	44.1	44.1	47.4	5.50	0.11	1.00	0.56
NDF								
Intake, kg/d	7.82	7.77	7.89	8.16	0.19	0.56	0.64	0.37
Omasal flow, kg/d	3.97	3.65	3.66	3.40	0.45	0.27	0.97	0.58
Apparent digestion, kg/d	3.84	4.13	4.23	4.88	0.45	0.05	0.71	0.08
Apparent digestion, % of NDF intake	49.2	52.6	53.0	59.8	5.83	0.16	0.92	0.22
ADF								
Intake, kg/d	5.29	5.10	5.16	5.25	0.20	0.49	0.75	0.73
Omasal flow, kg/d	2.44	2.19	2.35	2.06	0.36	0.30	0.52	0.34
Apparent digestion, kg/d	2.86	2.91	2.81	3.25	0.47	0.47	0.63	0.12
Apparent digestion, % of ADF intake	53.9	56.1	53.2	61.6	7.66	0.44	0.53	0.15

[1]Values are least squares means obtained from 4 cows.

Table 3.8. The influence of feeding oscillating dietary CP concentrations on intake, digestibility, and omasal flow of N constituents in dairy cows[1].

	Treatments					Contrast, *P* value		
Item	STATIC	OSC-24	OSC-48	OSC-72	SEM	STATIC vs. OSC	OSC-24 vs. OSC-48	OSC-48 vs. OSC-72
N intake, g/d	774.3	727.4	751.4	828.7	41.2	0.90	0.62	0.20
N apparent digested in the rumen								
g/d	-54.7	22.8	32.4	91.6	68.6	0.17	0.91	0.54
% of N intake	-7.01	3.43	4.09	11.7	9.40	0.18	0.95	0.55
N truly digested in the rumen								
g/d	494.6	502.0	530.2	572.5	45.1	0.42	0.63	0.54
% of N intake	64.0	69.3	70.9	68.7	3.57	0.15	0.70	0.65
Flow at omasal canal								
N								
g/d	829.0	704.6	719.1	712.3	74.6	0.06	0.81	0.92
% of N intake	107.0	96.6	95.9	88.3	9.40	0.18	0.95	0.55
NH_3-N, g/d	68.5	60.0	60.2	60.7	4.50	0.11	0.96	0.94
NAN[2]								
g/d	760.5	644.6	658.8	652.6	71.1	0.07	0.93	0.81
% of N intake	98.1	88.3	87.8	80.8	8.83	0.18	0.95	0.55
NANBN[3]								
g/d	211.3	165.4	161.0	192.5	22.2	0.07	0.83	0.22
% of NAN flow	27.0	26.1	24.1	28.1	1.49	0.56	0.30	0.10
% of N intake	27.1	22.5	20.9	23.9	2.94	0.14	0.65	0.46
% of DM intake	0.789	0.636	0.583	0.691	0.074	0.04	0.44	0.21
Total bacterial NAN								
g/d	549.3	479.2	497.8	462.9	52.9	0.12	0.68	0.52
% of NAN	73.0	73.9	75.9	71.9	1.49	0.56	0.30	0.10

[1]Values are least squares means obtained from 4 cows.

[2]NAN: non-ammonia-nitrogen

[3]NANBN: non-ammonia-non-bacterial-nitrogen

5. DISCUSSION

In the last couple of decades, there has been increasing pressure on the ruminant livestock sector, particularly intensive dairy and feedlot operations, to decrease N excretion into the environment. Therefore, there has been a significant focus on research directed at developing intervention strategies to improve the efficiency of N utilization in ruminants which, consequently, would reduce N excretion into the environment. It has been shown that N utilization can be increased by feeding crude protein in an oscillating regimen to steers (Cole et al., 2003; Archibeque et al., 2007b) and sheep (Cole, 1999; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011). To my knowledge, only one study has investigated the effects of feeding oscillating dietary CP concentrations to dairy cows and its effects on milk production (Brown, 2014), so there is a need for more studies to determine the effectiveness of this approach as a strategy to improve the efficiency of N utilization in ruminants.

A major mechanism that is partly responsible for the positive influence on N utilization of feeding oscillating dietary CP levels is urea-N recycling to the gastrointestinal tract, particularly the rumen (Cole, 1999). Urea-N that is secreted from the bloodstream into the rumen is rapidly degraded to NH_3-N by ureolytic (epimural) bacteria that are attached to the ruminal wall, thus contributing an important source of N for microbial growth (Krause et al., 2003). A tendency for an increased net flux of urea-N across the PDV was reported when an oscillating CP dietary regimen (9.5 and 15.5% CP oscillated on a 48-h basis) was fed compared with a static (12.5% CP) dietary regimen to sheep (Archibeque et al., 2007c). Similarly, Doranalli et al. (2011) reported an increased serosal-to-mucosal urea flux when sheep were fed an oscillating CP dietary regimen (10.3 and 16.1% CP) compared with a static dietary CP regimen (12.7% CP). Of the numerous factors that regulate urea-N entry into the rumen, elevated ruminal NH_3-N concentrations have been reported to have inhibitory effects on urea-N transfer into the rumen (Lu et al., 2014). Doranalli et al. (2011) showed that, in ruminal epithelial tissues mounted in Ussing chambers, greater rates of serosal-to-mucosal urea transfer were observed with tissues that were obtained from animals that were receiving a low CP diet compared to a high CP diet at the time of slaughter. To derive any potential benefits on N retention due to feeding oscillating dietary CP concentrations, Cole (1999) indicated that the oscillation frequency (i.e., changes in dietary CP) had to be in synchrony with retention time of digesta in the rumen. This would

ensure that greater rates of urea-N recycling with the low CP diet would occur when ruminal NH_3-N concentration was sub-optimal in terms of supporting microbial growth. One factor that regulates the retention time of digesta in the rumen is the level of DM intake. Because dairy cows are fed higher forage diets (around 50% of dietary DM) compared to finishing cattle (around 80-90% of dietary DM), it is plausible that the oscillation frequencies that have been used in research with finishing cattle might not be suitable for dairy cows due to differences in rumen retention time of digesta. Also, dairy cows consume more DM when compared with finishing cattle, and this might also influence rumen retention time of digesta. Brown (2014) used an oscillation frequency of 48 h for dairy cows which, presumably, was based on the published studies with finishing cattle. Therefore, the first major objective of my thesis research was to determine was the optimum frequency of feeding oscillating dietary CP (i.e., 24-, 48- or 72-h) in dairy cows fed a TMR. In this study, N intake did not differ in cows fed the OSC-48 diet compared to those fed the OSC-24 and OSC-72 diets. Also, total N excretion as a percentage of N intake tended to be lower in cows fed the OSC-48 diet compared to those fed the OSC-24 diet. Consequently, apparent N balance increased by approximately 41% when cows were fed the OSC-48 diet compared to the OSC-24 or STATIC diets. However, apparent N balance was similar when the OSC-72 diet was fed compared to the OSC-48 or STATIC diets. These results tend to suggest that feeding an oscillating diet on a 48-h regimen is the best regimen in order to improve N utilization in dairy cows.

Another objective of this study was to determine the effects of feeding oscillating CP diets on feed intake, milk production, and milk composition in dairy cows. There was no difference in DMI in cows fed the OSC diets compared with the STATIC diet. Similarly, when other studies compared oscillating dietary CP on a 48-h regimen to a static CP diet, there were no dietary effects on DMI in dairy cows (Brown, 2014), beef cattle (Cole et al., 2003; Archibeque et al., 2007a,b) and sheep (Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009). However, Doranalli et al. (2011) found a tendency for DMI to be lower with an oscillating CP dietary regimen (10.3 and 16.1% CP oscillated on a 48-h basis) was fed to sheep compared with a static CP diet (12.7% CP). Factors such as the extent of ruminal and total-tract DM digestion and rate of passage can influence DMI (Ingvartsen, 1994). In the present study, omasal outflow of DM, and ruminal and total-tract DM digestion were not influenced by dietary treatment; therefore, it is not surprising that DMI was similar across dietary treatments.

Milk production (mean = 36.4 kg/d) was not affected by dietary treatment. In comparison with the current study, Brown (2014) reported that milk production was similar in cows fed a static CP diet compared with an oscillating diet. Dry matter intake and milk yield are positively correlated (NRC, 2001), so it is not surprising that there was no dietary effect on milk production since DMI was similar across dietary treatments. Another factor that could influence milk production is post-absorptive nutrient supply through differences in diet digestion. In the present study, although ruminal digestion of DM, OM and NDF was greater or tended to be greater in cows fed the OSC diets compared to those fed the STATIC diet, total-tract nutrient digestibilities were not affected by dietary treatment This would suggest that nutrient supply to support milk production was similar across dietary treatments; consequently, no differences in milk production due to dietary treatment were observed. Ludden et al. (2003) and Archibeque et al. (2007a) reported that when finishing steers were fed an oscillating CP diet compared to a static CP diet, average daily gain (which is a measure of animal performance as milk yield is in the present study) was unaffected by dietary treatment. In contrast, Doranalli et al. (2011) reported that weight gain improved by 30% when oscillating CP diet were fed to sheep compared to a static CP diet.

Milk protein yield was greater in cows fed the STATIC diet as compared to those fed the OSC diets (1.22 vs. 1.14 kg/d). Brown (2014) reported that feeding an oscillating diet to dairy cows had no effect on milk protein yield, although cows fed the static diet had a numerically higher milk protein yield compared to those fed the oscillating CP diets which would support observations from the present study. Although cows fed the OSC diets had a greater apparent N balance when compared to those fed the STATIC diet, it appears that the greater N retention could have been directed towards body weight gain or other physiological functions rather than towards milk protein synthesis. In the present study, we did not monitor changes in body weight gain as that data is somewhat difficult to interpret with Latin square designs in which experimental animals are switched from one dietary treatment to the next. Milk content and yield of fat was not effected by feeding a STATIC diet compared with a OSC diet to cows. Similarly, Brown (2014) reported that feeding an oscillating diet to dairy cows had no effect on milk fat content and yield. When an OSC diet was fed to cows there was no effect on lactose content and yield compared with a STATIC diet which is in agreement with Brown (2014). In the present

study, total-tract nutrient digestibilities were not affected which could suggest that nutrient supply for milk fat and lactose were similar among treatments.

Both MUN and PUN concentrations were similar in cows fed the STATIC and OSC diets. Plasma urea-N concentrations can be used as a parameter to monitor protein status in ruminants, whereas MUN concentrations can be used as a parameter to monitor protein status and the efficiency of N utilization in lactating ruminants (Hammond, 1997). Milk urea-N concentration is highly correlated with PUN concentration because urea freely diffuses from the bloodstream into the milk (Baker et al., 1995; Butler et al., 1996; Roseler et al., 1993). Plasma urea-N concentration is highly correlated with ruminal NH_3-N concentration as ruminally-derived NH_3-N is the major substrate contributing one N towards hepatic ureagenesis (Hammond, 1983a; Hennessy and Nolan, 1988). In the present study, ruminal NH_3-N concentration was greater in cows fed the OSC diets compared to those fed the STATIC diet. As the absorption of ruminal NH_3-N into portal blood is a concentration-dependent process, it is rather surprising that PUN and, consequently, MUN concentrations were similar in cows fed the OSC compared to the STATIC diets. There was a tendency for MUN concentration to be higher in cows fed the OSC-48 diet compared to those fed the OSC-24 diet, and this response can be partly attributed to the greater ruminal NH_3-N concentration in cows fed the OSC-48 diet when compared to those fed the OSC-24 diet. Brown (2014) observed no difference in MUN concentration when cows were fed an oscillating CP diet compared with a static CP diet; however, it should be noted that dietary CP concentrations in that study were lower when compared to those that were used in the present study. The lack of effect of feeding the OSC diets on PUN concentration when compared to the STATIC diet is in agreement with other studies (Cole, 1999; Cole et al., 2003; Ludden et al., 2003; Archibeque et al., 2007b; Doranalli and Mutsvangwa 2009).

Another objective of this study was to determine the effects of feeding oscillating CP diets on ruminal fermentation characteristics (NH_3, SCFA and pH), microbial protein synthesis, ruminal outflow of nutrients, and N balance in dairy cows. Ruminal SCFA concentrations were not affected by feeding the OSC diets compared with feeding the STATIC diet. In contrast, others have reported increases in ruminal acetate (Ludden et al., 2002a; Doranalli et al., 2011), propionate (Doranalli et al., 2011), and total SCFA (Doranalli et al., 2011; Ludden et al., 2002a) concentrations when oscillating CP diets were fed to sheep compared with static CP diets. The

differences between studies might be because Ludden et al. (2002a) fed the sheep a high-forage diet and animals were feed-restricted, whereas Doranalli et al. (2011) fed the sheep a high-concentrate diet. Differences in levels of DMI could also partly account for the discrepancies between studies in the effects of feeding oscillating CP diets on ruminal SCFA concentrations. Collins and Pritchard (1992) observed that when wether lambs were supplemented with either soybean meal or corn gluten meal daily or every other day, there were no dietary effects on ruminal SCFA concentrations and pH. Ruminal pH was unaffected when OSC diets were fed to cows compared with STATIC diets, which is in agreement with other studies (Ludden et al., 2002a; Doranalli et al., 2011). The lack of effect of dietary treatment on ruminal pH and SCFA concentrations in this study could reflect that DMI was unaffected.

Greater ruminal NH_3-N concentrations were observed when cows were fed the OSC diets compared with a STATIC diet (12.7 vs. 11.6 mg/dL). Also, cows fed the OSC-48 diet had a greater ruminal NH_3-N concentration compared to those fed the OSC-24 and the OSC-72 diets. Doranalli et al. (2011) observed no difference in ruminal NH_3-N concentrations when sheep were fed high-concentrate diets with either an oscillating CP or static CP regimen. Ludden et al. (2002a) observed a tendency for ruminal NH_3-N concentrations to be lower when high-forage diets were fed on an oscillating regimen to sheep compared to a static diet. Beauty et al. (1994) observed that supplementing CP three times a week to steers fed wheat straw sustained high ruminal NH_3-N concentrations compared to daily supplementation. In the present study, mean ruminal NH_3-N concentrations across dietary treatments were well above 5 mg/dL which has been suggested as the minimum ruminal NH_3-N concentration for maximum MP synthesis (Satter and Slyter, 1974); however, cows fed the OSC-72 diet had ruminal NH_3-N concentrations that were below 5 mg/dL at some sampling time points during the feeding cycle when the 13.5% CP diet was fed. This could indicate that the 13.5% CP diet was somewhat deficient in supplying NH_3-N for MP synthesis at certain periods during the feeding cycle; however, this is unlikely as microbial NAN flow at the omasal canal was similar across dietary treatment. When ruminal NH_3-N supply is deficient (possibly at times during the feeding cycle with the 13.5% CP diet in the present study) due to a lower dietary N intake, ruminants become more dependent on urea-N that is transferred from the bloodstream into the rumen as a source of N for microbial growth (Lapierre and Lobley, 2001). There are many factors that can influence the amount of urea-N that is recycled back into the rumen; among these factors, elevated ruminal NH_3-N

concentrations have been demonstrated to have inhibitory effects on urea-N transfer from the bloodstream into the rumen (Kennedy and Milligan, 1980; Lu et al., 2014). Although whole-body urea-N kinetics were not measured in the present study, it is plausible that there was greater urea-N transfer from the bloodstream into the rumen when cows fed the OSC diets were receiving the 13.5% CP diet. When the STATIC diet was fed to the dairy cows compared to those fed the OSC diets total N flow at the omasal canal were higher than the amount of N that was consumed by the cows which can indicate that some N was recycled back into the rumen through urea-N recycling. A potential shortcoming of the present study is that the actual dietary CP concentrations deviated from the targeted dietary CP concentrations, largely due to variations in forage CP content. For the OSC diets, the target CP concentrations were 13.5 and 19.5% CP, but the actual CP concentrations were 14.3 and 20.3 respectively. Therefore, it is plausible that that the low (14.3% CP) diet might not have been deficient in CP as had been planned. Also, the 20%? CP diet could have over-supplied N. Collectively, these shortcomings in actual dietary CP composition could have negated any benefits that could potentially have been derived from feeding the OSC dietary regimen. With greater rates of urea-N recycling to the rumen, we typically observe greater post-ruminal N flows when compared to N intakes (Broderick et al., 2010). The fact that there was a tendency for total N flow at the omasal canal for cows fed the STATIC diet compared with the cows fed the OSC dietary regimens implies that quantitative rates of urea-N transfer to the rumen were higher for the STATIC.

A major contribution of this thesis research is elucidating the effects of oscillating dietary CP levels on ruminal digestion, MP synthesis, and post-ruminal nutrient flow. To my knowledge, this is the first study that has taken that approach. Limited research with sheep (Ludden et al., 2002a) has studied the impacts of feeding oscillating dietary CP levels on ruminal digestion and nutrient flow. In the present study, when the STATIC diet was fed to cows it had a tendency to increase DM and OM omasal flows compared with the OSC diets. Apparent ruminal DM digestion was lower for cows fed the STATIC diet compared to the cows fed the OSC diets; similarly, apparent ruminal OM digestion was lower in cows fed the STATIC diet compared to those fed the OSC diets. Ludden et al. (2002a) found no difference in apparent ruminal OM digestion when a high-forage oscillating diet was fed to sheep on a 48-h regimen (13.2 and 16.7% CP) compared to a static diet (15% CP). In the present study a faster rate of DM and OM omasal flow could have led to the decrease in apparent ruminal DM and OM digestion because

rumen retention time decreased. In this study, feeding the OSC diets compared to the STATIC diet did not affect apparent total-tract digestibility of nutrients; however, because apparent ruminal digestion of DM and OM were altered by dietary treatment it suggests that the site of nutrient digestion was shifted. Ludden et al. (2002a) reported no difference in OM disappearance in the small intestine and the hindgut when a high-forage oscillating diet was fed to sheep on a 48-h regimen compared to a static diet. Apparent ruminal NDF digestion was higher in cows fed the OSC diets compared to those fed the STATIC diet, but apparent ruminal ADF digestion was not affected by treatment. It has been reported that feeding an oscillating diet compared to a static diet to sheep had no effect on NDF and ADF apparent ruminal digestion (Ludden et al., 2002a).

Dietary treatments had no effect on apparent total-tract digestibilities. When comparing oscillating 48-h dietary CP regimen to the static dietary CP diet, other studies observed that there was no dietary effect on total-tract digestibility of DM (Cole 1999; Ludden et al., 2002b; Archibeque et al., 2007a,b; Doranalli and Mutsvangwa, 2009), OM (Ludden et al., 2002a; Archibeque et al., 2007a,b; Doranalli and Mutsvangwa, 2009), N (Cole 1999; Cole et al., 2003; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009), NDF and ADF (Ludden et al., 2002a,b). In contrast, when comparing oscillating 48-h dietary CP regimens to static dietary CP diets, other studies found that total-tract digestibility of N (Archibeque et al., 2007b), and NDF and ADF (Doranalli and Mutsvangwa, 2009) were higher in animals fed the oscillating diet compared to those fed the static diet. The higher NDF and ADF total-tract digestibility that was observed by Doranalli and Mutsvangwa (2009) when the oscillating diet was fed to sheep might have been due to an increase in urea-N recycling to the rumen which, consequently, could have improved microbial proliferation in the rumen. In that study, bacterial NAN flow increased when the oscillating diet was fed compared with the static diet, which would indicate a greater microbial proliferation in the rumen.

Nitrogen that was apparently or truly digested in the rumen was unaffected by diet. Nitrogen flow at the omasal canal tended to be greater in cows fed the STATIC diet compared to those fed the OSC diets. Ludden et al. (2002a) reported no difference in ruminal apparent N disappearance and N flow to the small intestine when an oscillating diet was fed compared with a static diet. When the STATIC diet was fed to the dairy cows more N was digested in the rumen than the amount that was consumed by the cows. This could indicate that there was some N that

was recycled back into the rumen through urea-N recycling. Although some studies have reported that feeding oscillating CP diets to sheep increased urea-N recycling (Archibeque et al., 2007c; Doranalli et al., (2011), it appears that this was not the case in the present study because the amount of N digested in the rumen when oscillating diets were fed to cows did not exceed the amount that was consumed by the cows. Archibeque et al. (2007c) found evidence that an increased uptake of urea-N to the portal-drained viscera (PDV) might be part of the mechanism that increases N retention. In that study, the venous-arterial difference technique was used to investigate the net flux of nitrogenous compounds across the PDV in growing wethers when an oscillating diet (9.5% and 15.5% CP) was fed compared with a static (12.5% CP) diet. In that study, there was a tendency for a greater net flux of urea-N across the PDV in animals fed the oscillating CP diet compared to the static diet (even though N intakes were similar across diets). Also, Doranalli et al. (2011) reported an increase in the serosal-to-mucosal urea flux when sheep were fed the oscillating (10.3 and 16.1% CP) diet when compared to those fed the static diet (12.7% CP).

Non-ammonia-nitrogen tended to be higher for cows fed the STATIC diet compared to the cows fed the OSC diets. Non-ammonia-non-bacterial-nitrogen tended to be greater (by 22%) in the cows fed the STATIC diet compare to the cows fed the OSC diets. Non-ammonia-non-bacterial-nitrogen as a % of DMI were higher for cows fed the STATIC diet compared to the cows fed the OSC diets (0.789 vs 0.637 g/d). There was no effect on total bacterial NAN flow when the STATIC diet was fed to cows compared with the oscillating diets. Total bacterial NAN flow as a % of NAN flow tended to be higher in cows fed the OSC-48 compared with cows fed the OSC-72 diet. No effect on total bacterial N flow was reported when an oscillating diet was fed to sheep compared with a static diet (Ludden et al., 2002a). In contrast, a tendency for higher bacterial NAN flow were estimated when sheep were fed an oscillating diet compared to a static diet (Doranalli and Mutsvangwa, 2009).

Nitrogen intake was not affected when the OSC diets were fed compared with the STATIC diet. Similar results were reported when an oscillating diet was fed to dairy cows (Brown, 2014), beef cattle (Cole et al., 2003; Archibeque et al., 2007a,b), and sheep (Cole, 1999; Ludden et al., 2002a,b; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011) compared to a static diet. Urinary and fecal N outputs were unaffected when OSC diets were fed to cows compared with the STATIC diet. No effect on urinary N (Cole 1999; Ludden et

al., 2002b; Cole et al., 2003; Archibeque et al., 2007b, c) and fecal N (Ludden et al., 2002b; Cole et al., 2003; Archibeque et al., 2007c) outputs were reported when an oscillating diet was fed compared with a static diet. In contrast, Archibeque et al. (2007b) and Doranalli et al. (2011) found that feeding a static CP diet increased fecal N output by 11 to 16% compared with feeding an oscillating CP diet. Doranalli and Mutsvangwa (2009) and Doranalli et al. (2011) also observed that, by feeding a static CP diet, urine N output increased by up to 48% when compared to feeding an oscillating CP diet in sheep. When feeding oscillating dietary CP levels, the available evidence indicates that N utilization is improved when the low CP diet is deficient and the high CP diet is adequate in terms of meeting the metabolizable protein requirements of the animal (Cole, 1999). In the present study, there was a 22.5% increase in apparent N balance when cows were fed the OSC diets compared with the STATIC diet (195.3 vs 159.4 g/d), although there were no differences in N intake and total N output. This response can be partly attributed to a numerically higher N intake and numerically lower N outputs when the OSC diets were fed compared with the STATIC diet. Therefore, these data tend to suggest that we can increase the efficiency of N utilization in dairy cows by feeding oscillating CP diets. Nitrogen retention as a percentage of N intake was increased when lambs were supplemented with SBM or corn gluten meal every other day compared with daily supplementation (Collins and Pritchard, 1992). In studies, in which high-concentrate diets were fed on a 48-h oscillation regimen compared to a static regimen, N retention was increased by up to 68% in beef cattle (Cole et al., 2003; Archibeque et al., 2007b) and sheep (Cole, 1999; Archibeque et al., 2007c; Doranalli and Mutsvangwa, 2009; Doranalli et al., 2011). In contrast, Ludden et al. (2002b) did not find that by feeding a high-forage oscillating CP diet to sheep improved N retention when compared with a static diet. However, the CP% in the oscillating diets fed were relatively higher (13 and 17%) than in other studies (10.0 and 15.0% CP, Cole, 1999; 9.9 and 14.2% CP, Archibeque et al., 2007c; 9.5 and 15.5% CP, Doranalli and Mutsvangwa, 2009; 10.3 and 16.1% CP, Doranalli et al., 2011), which might have decreased N utilization. On the other hand, by feeding a high-forage diet this can result in the inadequate supply of ruminally-fermentable carbohydrate which, in turn, can restrict microbial growth due to the limited availability of energy (Egan et al., 1986).

6. GENERAL DISCUSSION

There is increasing public pressure on intensive dairy operations to reduce nitrogen (N) excretion into the environment. This thesis research investigated the potential for feeding oscillating dietary CP concentrations as a strategy to improve the efficiency of N utilization in dairy cows, which could reduce the environmental impact of dairy farms.

Based on the results from my study, feeding an oscillating CP diet on a 48-h regimen is the optimum frequency to use in dairy cows fed a TMR because total N excretion as a percentage of N intake tended to be lower in cows fed the OSC-48 diet compared to those fed the OSC-24 diet. Also apparent N balance increased by approximately 41% when cows were fed the OSC-48 diet compared to the OSC-24 or STATIC diets. Work with beef cattle and sheep had established that a 48-h oscillation frequency was optimum in terms of improving N utilization; however, it was important to determine what the optimum frequency is in dairy cows as the diets that they are fed, their levels of DMI, and rates of passage differ when compared to beef cattle and sheep. Therefore, establishing that a 48-h oscillation frequency can be recommended for use in dairy cows is an important contribution of this thesis research, and this recommendation can be applied for future research studies in this area.

A potential area that warrants investigation is the oscillation of dietary RDP, rather than dietary CP and its effect on milk production and composition, ruminal fermentation, ruminal and total-tract nutrient digestion and N balance. Studies have shown that by changing the amount of RDP (low vs. high) fed to animals can alter ruminal NH_3-N concentrations and thereby effect N utilization (Section 2.4.2). For the next potential study treatments should include: 1) a static diet containing adequate RDP; 2) a static diet containing defiecient RDP; 3) oscillation between diets containing a low and high CP content on a 48-h regimen both containing adequate RDP; 4) oscillation between diets containing a low and high CP content on a 48-h regimen both containing defieicnt RDP. By combining a 48-h oscillation regimen with the amount of dietary RDP supply to the animal, the proposed study would generate more knowledge on how to improve N utilization in dairy cows and thereby improve environmental stewardship.

One of the objectives of this study was to investigate the effect of feeding oscillating dietary CP to dairy cows as a strategy to improve production and N utilization. Based on the results from my study, feeding an OSC CP diet to dairy cows does not improve production compared with a STATIC diet but it improves N utilization. Although N excreted was not

affected when an OSC-48 diet was fed to cows compared to a STATIC diet, cows fed the OSC-48 diet excreted 29.6 g/d less N than the cows fed the STATIC diet. This would mean that in a standard lactation (i.e., 305 d) a dairy cow fed the OSC-48 diet would excrete approximately 9 kg less N than a dairy cow fed the STATIC diet. In Saskatchewan, the dairy cow population is approximately 26,900 (Statistics Canada, 2015). If an OSC-48 diet were fed to the cows in Saskatchewan, approximately 242,853 kg less N would be excreted annually compared with cows fed a STATIC diet. I conclude that, even though there were no production benefits (DMI or milk production) when OSC CP diets were fed to cows, there was an improvement in environmental stewardship.

7. GENERAL CONCLUSION

In this study the results demonstrate that feeding oscillating dietary CP diets to dairy cows on a 48-h basis improves N efficiency by enhancing N retention without compromising production when compared to feeding a STATIC CP diet or an oscillating dietary CP on a 24-h or 72-h basis. Thereby feeding dairy cows a CP diet on an oscillating 48-h regimen can be used as a strategy to increase N utilization thereby improving the impact on the environment.

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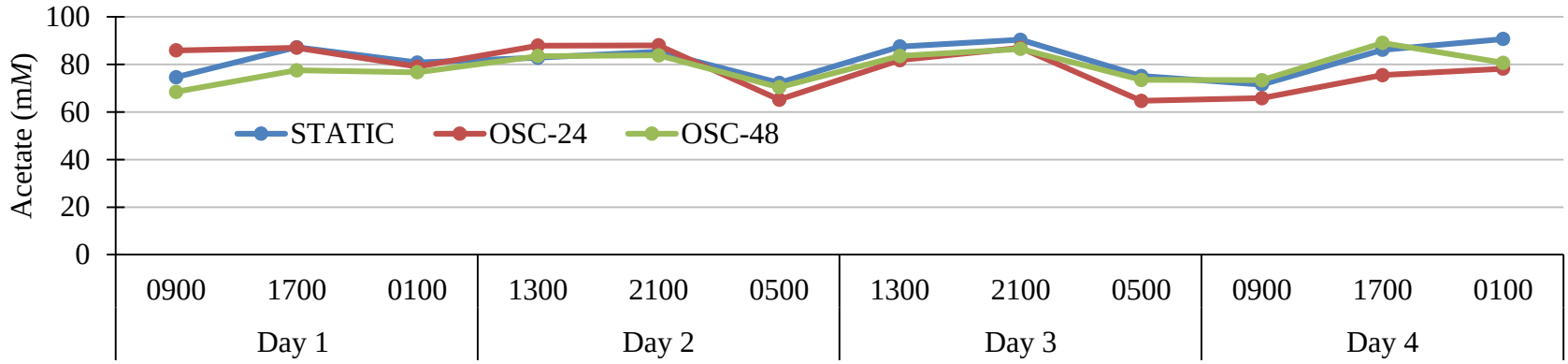
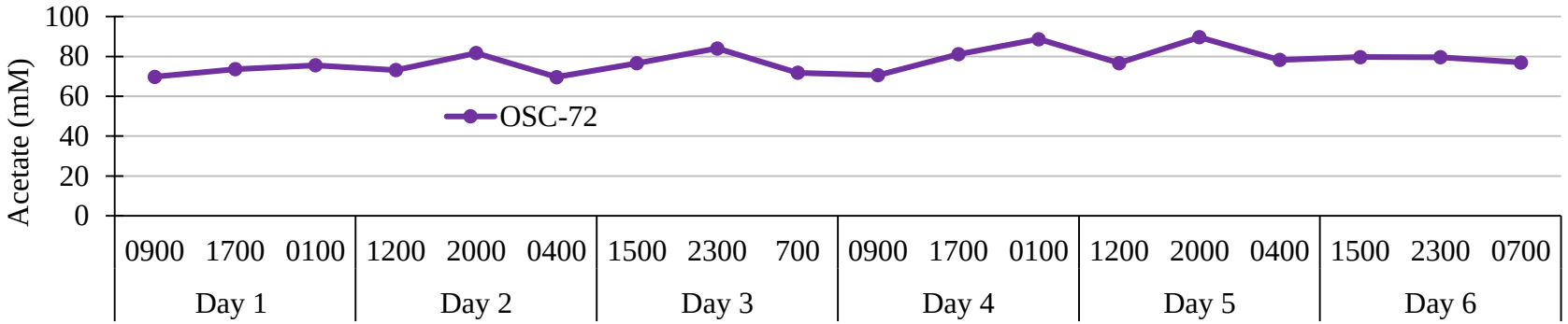
9. APPENDICES

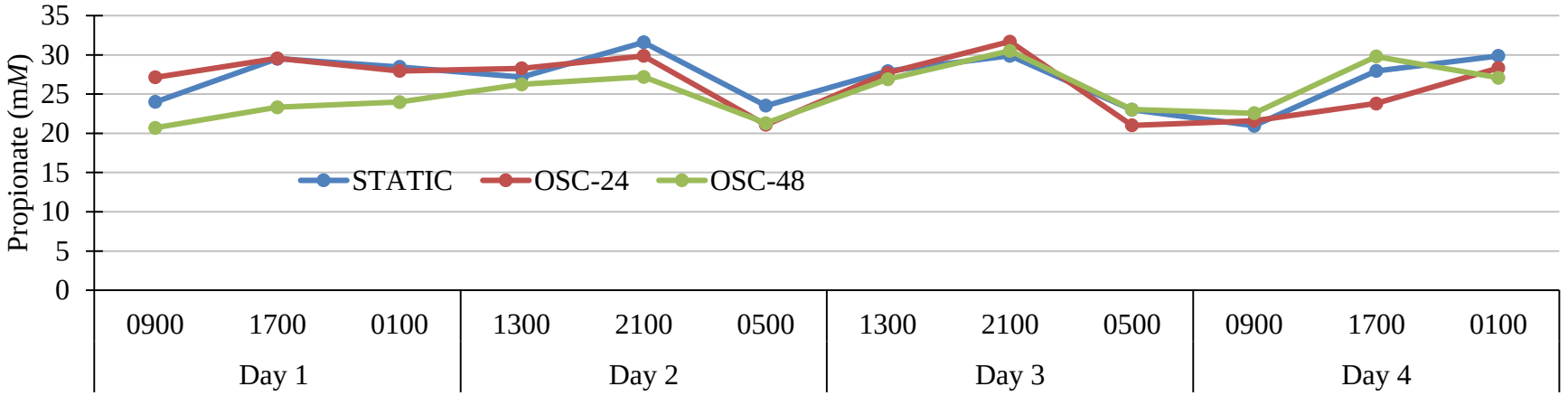
9.1 Appendix Figures



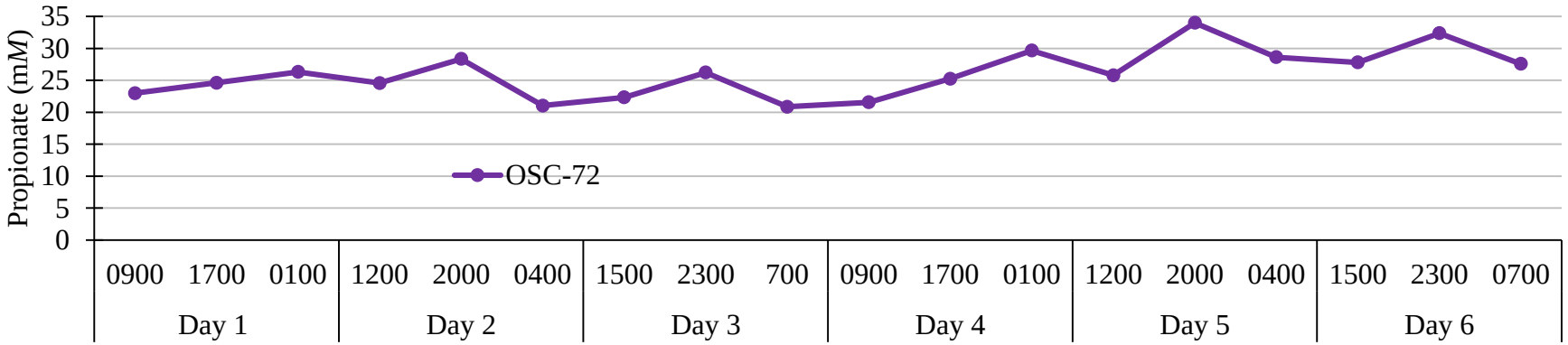
Appendix Figure 1a. The influence of feeding a STATIC, OSC-24 and OSC-48 diet on ruminal acetate concentrations in dairy cows. The cows on the OSC-24 diet were fed the 13.5% CP diet on d 2 and d 4 and the 19.5% CP diet on d 1 and d 3. The cows on the OSC-48 diet were fed the 13.5% CP diet on d 1 and d 2 and the 19.5% CP diet on d 3 and d 4. All cows were fed twice daily at 0900 and 1700 h.



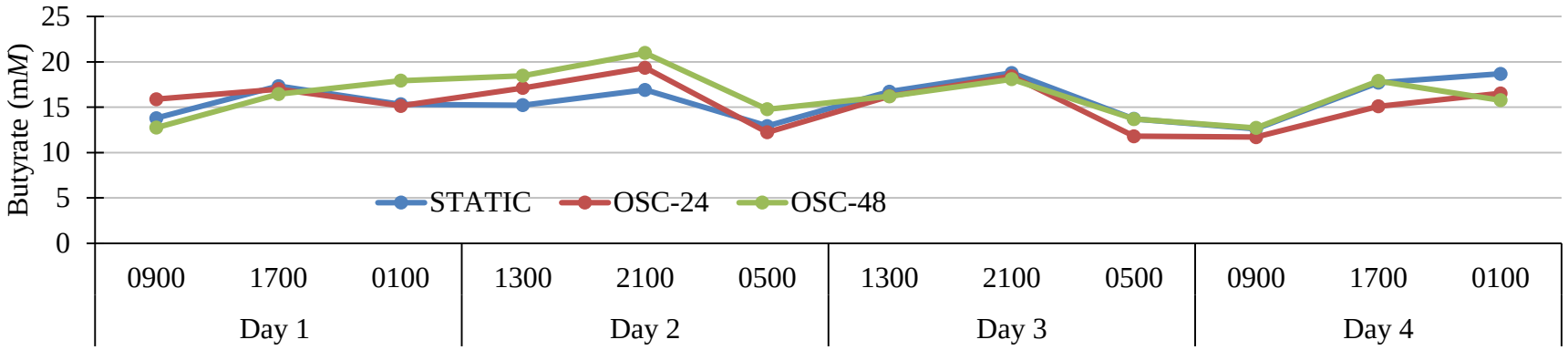
Appendix Figure 1b. The influence of feeding OSC-72 on ruminal acetate concentrations in dairy cows. The cows on the OSC-72 diet fed the 13.5% CP diet on d 4, 5 and 6 and the 19.5% CP diet on d 1, 2 and 3. All cows were fed twice daily at 0900 and 1700 h.



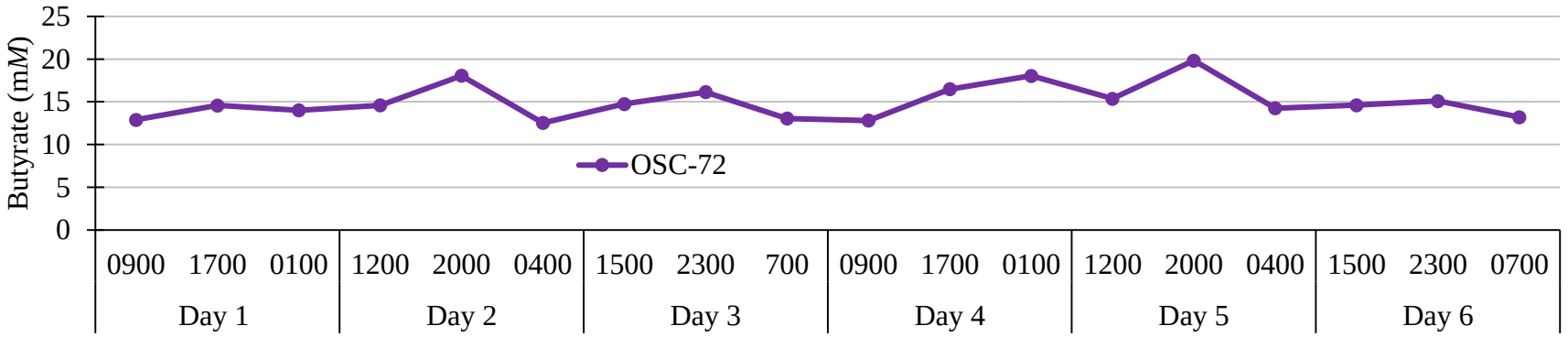
Appendix Figure 2a. The influence of feeding a STATIC, OSC-24 and OSC-48 diet on ruminal propionate concentrations in dairy cows. The cows on the OSC-24 diet were fed the 13.5% CP diet on d 2 and d 4 and the 19.5% CP diet on d 1 and d 3. The cows on the OSC-48 diet were fed the 13.5% CP diet on d 1 and d 2 and the 19.5% CP diet on d 3 and d 4. All cows were fed twice daily at 0900 and 1700 h.



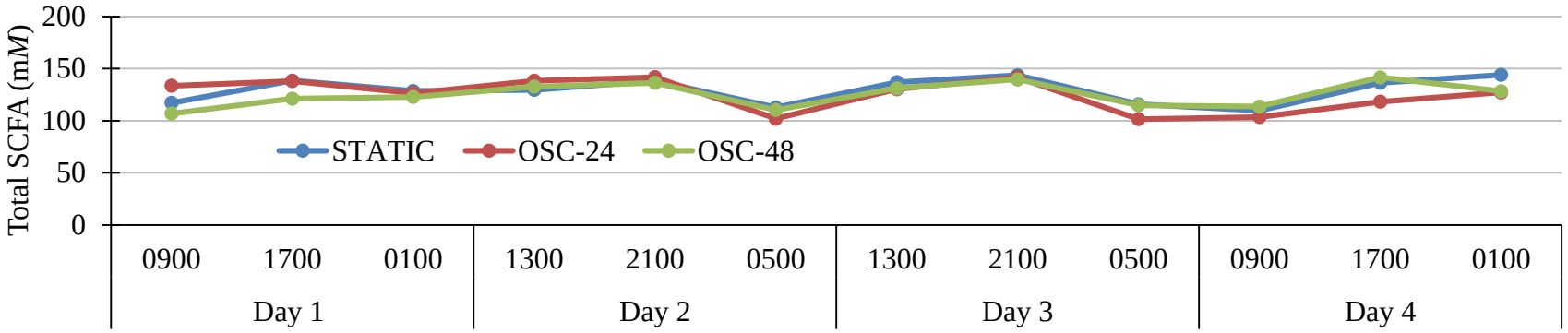
Appendix Figure 2b. The influence of feeding OSC-72 on ruminal acetate concentrations in dairy cows. The cows on the OSC-72 diet fed the 13.5% CP diet on d 4, 5 and 6 and the 19.5% CP diet on d 1, 2 and 3. All cows were fed twice daily at 0900 and 1700 h.



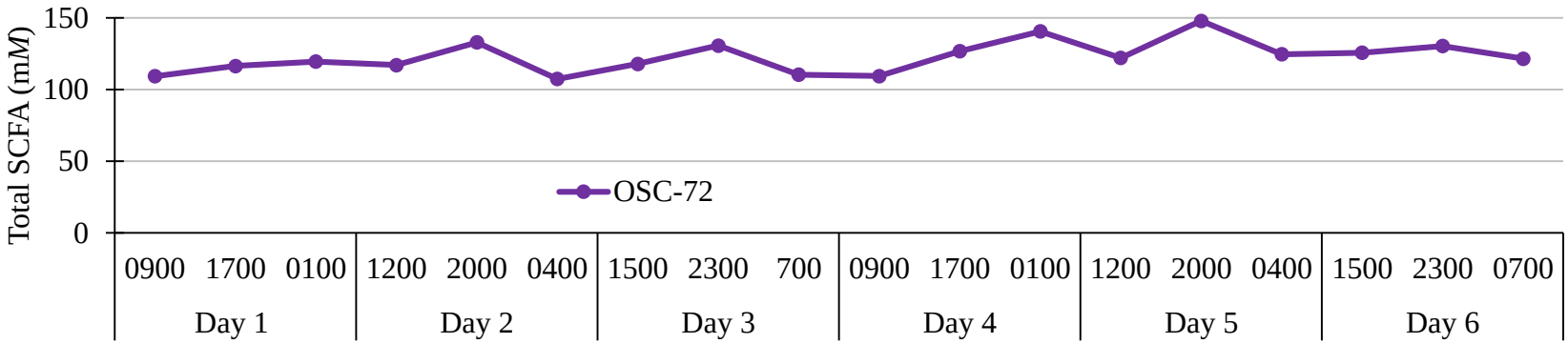
Appendix Figure 3a. The influence of feeding a STATIC, OSC-24 and OSC-48 diet on ruminal butyrate concentrations in dairy cows. The cows on the OSC-24 diet were fed the 13.5% CP diet on d 2 and d 4 and the 19.5% CP diet on d 1 and d 3. The cows on the OSC-48 diet were fed the 13.5% CP diet on d 1 and d 2 and the 19.5% CP diet on d 3 and d 4. All cows were fed twice daily at 0900 and 1700 h.



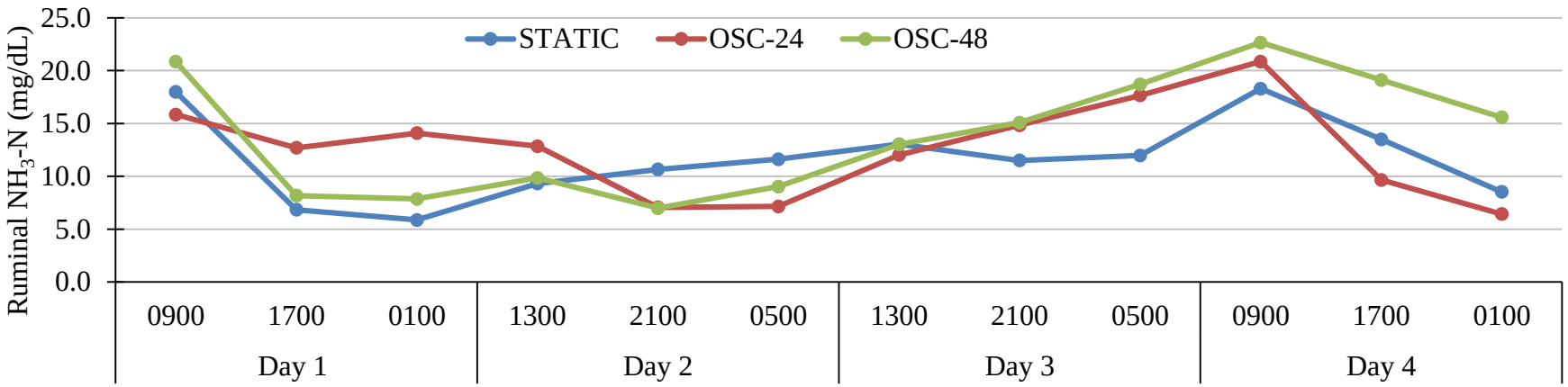
Appendix Figure 3b. The influence of feeding OSC-72 on ruminal butyrate concentrations in dairy cows. The cows on the OSC-72 diet fed the 13.5% CP diet on d 4, 5 and 6 and the 19.5% CP diet on d 1, 2 and 3. All cows were fed twice daily at 0900 and 1700 h.



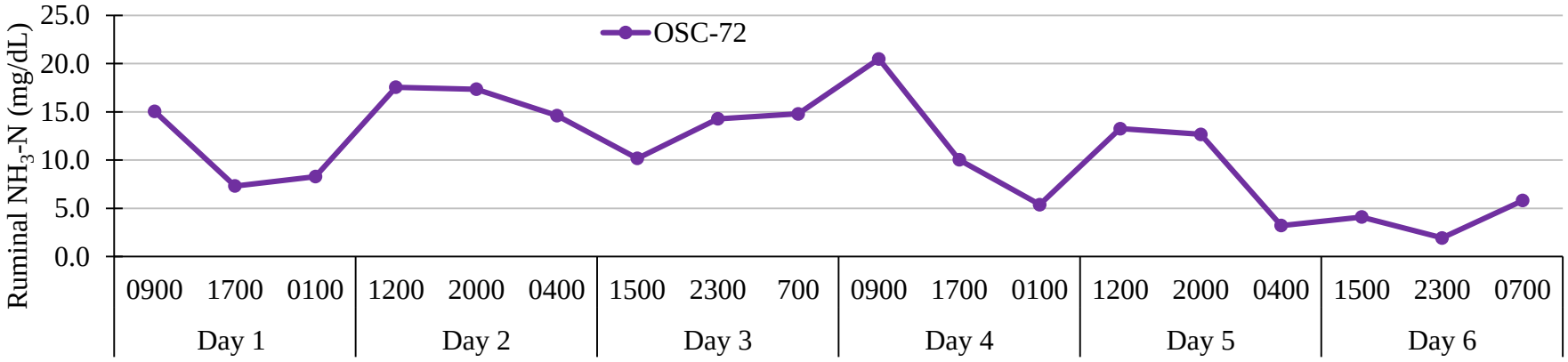
Appendix Figure 4a. The influence of feeding a STATIC, OSC-24 and OSC-48 diet on ruminal total SCFA concentrations in dairy cows. The cows on the OSC-24 diet were fed the 13.5% CP diet on d 2 and d 4 and the 19.5% CP diet on d 1 and d 3. The cows on the OSC-48 diet were fed the 13.5% CP diet on d 1 and d 2 and the 19.5% CP diet on d 3 and d 4. All cows were fed twice daily at 0900 and 1700 h.



Appendix Figure 4b. The influence of feeding OSC-72 on ruminal total SCFA concentrations in dairy cows. The cows on the OSC-72 diet fed the 13.5% CP diet on d 4, 5 and 6 and the 19.5% CP diet on d 1, 2 and 3. All cows were fed twice daily at 0900 and 1700 h.



Appendix Figure 5a. The influence of feeding a STATIC, OSC-24 and OSC-48 diet on ruminal NH_3-N concentrations in dairy cows. The cows on the OSC-24 diet were fed the 13.5% CP diet on d 2 and d 4 and the 19.5% CP diet on d 1 and d 3. The cows on the OSC-48 diet were fed the 13.5% CP diet on d 1 and d 2 and the 19.5% CP diet on d 3 and d 4. All cows were fed twice daily at 0900 and 1700 h.



Appendix Figure 5b. The influence of feeding OSC-72 on ruminal NH_3-N concentrations in dairy cows. The cows on the OSC-72 diet fed the 13.5% CP diet on d 4, 5 and 6 and the 19.5% CP diet on d 1, 2 and 3. All cows were fed twice daily at 0900 and 1700 h.